MORPHOLOGICAL AND HISTOCHEMICAL STUDIES ON FASCIOLA GIGANTICA COBBOLD, 1855

(ABSTRACT)

THESIS PRESENTED FOR THE DEGREE OF
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The present thesis, comprising seven chapters, pertains to comprehensive morphological and histochemical studies on the giant liver-fluke, Fasciola gigantica which has a fairly high incidence in the beef cattle of the agrarian tropics.

Apart from the introduction, historical review, and material and methods the other seven chapters are related to the I the tegument, II the parenchyma, III the digestive system, IV myoarchitecture, V the nervous system and the neurosecretory cells, VI the excretory system and the osmoregulation, and VII the reproductive system, respectively. The last one pertains to a concise bibliography comprising 155 references,cited in the text; only 9 of these references could not be consulted in original. Such titles are marked with an astarik. The plates (I - XXX) contain 196 figures and the text also incorporates 6 tables relating to histochemical observations.

As regards the tegument and the parenchyma it's salient morphological features of the respective regions and their cellular components have been described in detail, in the perspective of the ultrastructural studies made in recent years on closely related species. Similar studies have been made on the digestive system and detailed histological and functional aspects of the gastrodermis have been described in detail.

Detailed account of the arrangement of different patterns of musculature, nervous system and excretory system are also given

in successive chapters and emphasis has been accorded to the presence of nuclei in muscle bundles and the occurrence and types of the neurosecretory cells in various body regions.

The account of the reproductive system elaborates the male and female components and gonadal anomalies observed during the course of these studies have also been reported.

Though all these studies were basically carried out on morphological background, these have been concurrently substantiated with standard histochemical studies which has enabled the author to present an elaborate account of the functional morphology of this parasite. A correlation between seasonal factors, mainly rainfall and incidence of the parasite F. gigantica has also been noted and on these parameters may be utilized as a tool for monitoring a priori prospects relating to population dynamics to the incidence of F. gigantica in a particular locality.

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INTRODUCTION

Fasciola gigantica Cobbold, 1855, the giant liver-fluke of cattle is one of the most important trematode parasites of live-stock in the agricultural tropics and is of considerable importance from patho-biological as well as economic points of view because it renders tons of beef liver useless and costs much to the cattle industry.

Except a few epizootological studies, a country-wide epidemiological data regarding the incidence of this parasite is still wanting and the quantum of financial loss to the cattle industry of India is yet to be ascertained. This fluke is quite common in tropical and subtropical regions and appears to have a fairly high incidence in India, particularly in Bihar, Orissa, Punjab, Haryana and Uttar Pradesh, and Roy (1954) has reported as high an incidence as 75% in Kalimpong and Darjeeling regions. Similar data from other states of India is however, wanting and a study in this respect will certainly be pertinent as well as necessary.

In certain regions, including the Indian sub-continent, the distribution of F. gigantica also overlaps that of F. hepatica. Such types of mixed infections have been reported from Pakistan (Kendall, 1954), Thailand (Dissamarn, 1955), Turkmenia, USSR (Kibakin, 1961) and Japan (Watanabe,

1958), although such reports are not available from mainland India.

Besides causing liver-rot or fascioliasis, F. gigantica is also reported to be responsible for causing "Black disease" in association with the bacterium, Clostridium oedematiens, which produces complicated pathological syndromes in cattle. Apart from cattle ailments, human infections with F. gigantica have also been frequently reported yearly.

At Aligarh abbatoir, where buffaloes are brought mostly from adjoining areas which have a good irrigation drainage, a random fortnightly survey, conducted from January, 1975 through December 1980, has indicated an incidence to the extent of 12%, ranging between 2.6% in post-monsoon months and 11.6% during pre-monsoon summer following drought periods (Table I). A marked decline in the incidence during 1979 and 1980 has been correlated with the low rainfall in 1978 and almost drought conditions in 1979 (Fig. 1). However what seems more important from patho-biological point of view is the worm burden in an infected host rather than the number of cattle heads infected in a sample.

With augmented irrigational facilities and increasing snail populations, fascioliasis is likely to become more wide spread and serious a problem in the plains of Uttar Pradesh and may take to the same pattern as has been reported

by Patnaik (1971) from Orrisa.

The present study deals with preliminary morphological and histochemical investigations related to various systems of F. gigantica.

TABLE - I

Year	Max. % of infection	Max. worm burden flukes/head	Total rainfall in cm
1975	5.3	1700	104.9
1976	7.2	83	100.3
1977	8.4	92	114.7
1978	11.6	99	75.5
1979	3.3	80	41.1
1980	2.6	31	78.5

Fig. 1. Showing yearly total rainfall in cm and maximum yearly percentage infection of F. gigantica in buffaloes at Aligarh, based on random fortnightly survey, conducted from January, 1975 through December, 1980.

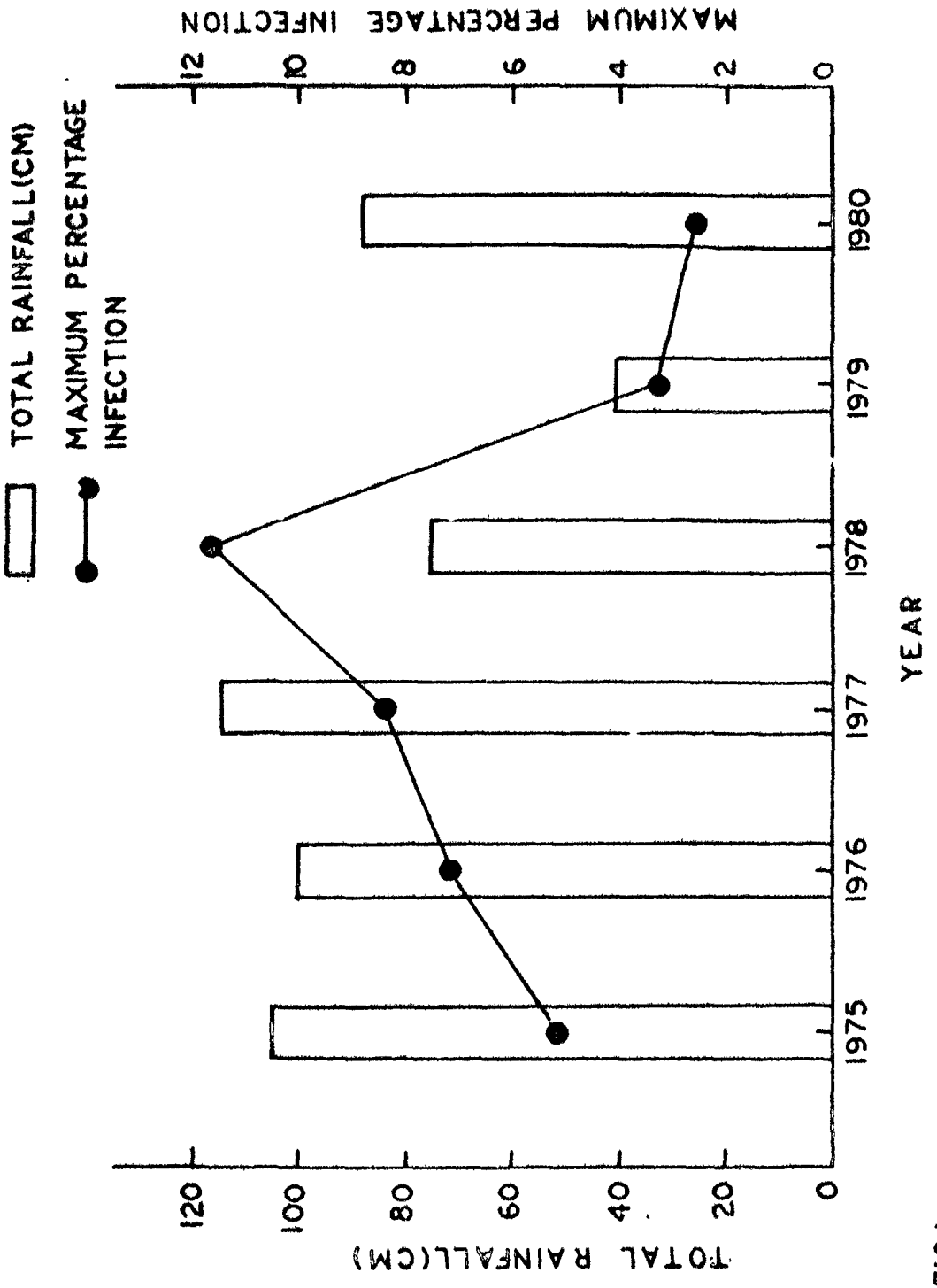
TOTAL RAINFALL(CM)
MAXIMUM PERCENTAGE INFECTION
TOTAL RAINFALL(CM)
MAXIMUM PERCENTAGE INFECTION
0
20
40
60
80
100
120
0
2
4
6
8
10
12
1975
1976
1977
1978
1979
1980
YEAR

FIG.1

MATERIAL AND METHODS

Live specimens obtained from the common bile ducts and gall bladders of infected hosts (buffaloes) were first washed with normal saline and brought to the laboratory in Hedonfleig's solution (Clegg, 1957) in thermos containers. These were subsequently fixed in neutral formalin 10%, formalin 4%, Helly's fluid, Bouin's fluid and Carnoy's fixatives for histological and histochemical studies. The material for histochemical preparation for esterases was fixed in cold acetone (Gomori, 1952) and for toto preparations, indicating the enzyme, specimens were fixed in cold 10% neutral formalin. Paraffin processed material, sectioned at 5-8 µm was stained with H & E, Mallory's triple stain and Heidenhain's Azan. Toto mounts were stained with Grenacher's Borax carmine. The specimens for toto mounts were stretched between two slides for the maximum display of the organ systems.

For qualitative histochemical studies various techniques, as given in Pearse (1960), Lillie (1965) and for standeradization of staining methods, those as cited in BDH manual (1972) were adopted. A schedule of histochemical techniques for relevant tests is given below (Table II).

TABLE - II

Substance investigated	Fixative	Technique employed
1. Glycogen and other polysaccharides	Carnoy's	PAS
2. "	"	PAS, after diastase digestion.
3. Glycogen	"	'Best's Carmine'
4. "	"	'Best's Carmine after diastase digestion.
5. Acid mucopoly saccharides	Bouin's	Alcian blue
6. Proteins	Carnoy's	Mercury bromophenol blue
7. Basic proteins	"	Acid solochrome Cyanine
8. RNA + DNA	"	Pyronine Y + Methyl green
9. Reticulin	Helly's	Silver impregnation method.
10. Lipids	Formalin 10%	Sudan Black B
11. Bound Lipids	Carnoy's	Acetone Sudan Black B
12. Non-specific esterases	10% Cold Neutral formalin and cold acetone	Indoxyl acetate
13. Acetylcholinesterase	10% Cold neutral formalin and cold acetone	Indoxyl acetates, after Eserine treatment
14. Acetyl cholinesterase	10% Cold neutral formalin and cold acetone	Acetylthiocholine iodide

The various histological, histochemical and in toto preparations were mounted in Canada balsam, DPX and glycerine jelly accordingly. For most of the morphological descriptions Camera lucida diagrams were drawn.

HISTORICAL REVIEW

Fasciola gigantica was first described by Cobbold (1855) from the liver of Giraffa camelopardalis and was subsequently referred to as Cladocoelium gigantеum by Stossich (1892). Jackson (1921) reviewed the various species and gave a comprehensive account of the gross morphology of various forms. Varma (1953) also studied this parasite and described an identical form from the liver of goat and buffaloes and named it as Fasciola indica but Sarwar (1957) ultimately regarded it as a synonym of F. gigantica and this view is now widely held.

Thapar and Tandon (1952) elucidated the life-history of this parasite at Lucknow, India, and found Lymnaea acuminata and L. auricularia as the common intermediate hosts. In recent years Patnaik (1971) furnished a comprehensive account of the autecology and synecology of this parasite as well as its intermediate hosts in Orissa.

Inspite of wide distribution in tropics and subtropics, little work seems to have been done on it's morphology. Except a few morphological studies like those of Bhalerao (1935), Watanabe and Iwata (1956 & 1958), Watanabe and Ueno (1959 & 1960 a,b), Rao and Madhavi (1962) and Shyamasundari & Rao (1975) on the anatomical features of its larval forms and few

morphological aspects, extensive studies on the morphology of F. gigantica are still wanting. Most of the studies are either epizootological, ecological or empirical and quantitative or related to it's chemotherapy.

F. hepatica, being the commonest and most abundantly available fluke, attracted attention of several workers and became an ideal tool and model of trematode morphology and physiology. Pioneer studies by early workers in this regard are those of Leuckart (1881 & 1886), Thomas (1881, 1882 & 1883 a,b) and Bettendorf (1897). Weinland and von Brand (1926) first set pace on histochemical and physiological aspects and elucidated the localization of glycogen in such parasites. von Brand & Mercado (1961) elaborated such studies and these were followed by Pantelouris (1964) and Halton (1967 a,b).

On F. gigantica preliminary physiological studies have been made by Goil (1958a,b & 1961) on carbohydrate, protein and lipid metabolism, and in recent years notable contribution have been made by Siddiqi & Lutz (1966) on it's ionic and osmotic regulation, and Lutz & Siddiqi (1967) on the nature of haemoglobin of this parasite vis-a-vis the same moiety of it's host.

Compared to F. gigantica, much work has been done on it's closest congener, F. hepatica and has formed the basis of generalization of trematode morphology and, in certain respects, trematode physiology, notable among these being

the studies of Prenant (1922), Muller (1923), Bugge (1929), Stephenson (1947a,b,c & d), Alvarado (1951) and Dawes (1954, 1962 & 1963a).

The advent of electron microscopy, and more recently that of scanning electron microscopy have opened new vistas in morphology and many workers utilized these tools in studying various aspects of morphology, and, among other structures, tegument and parenchyma first attracted attention of such workers, most significant among these being those of Senft (1959), Threadgold (1963 a,b), Threadgold & Gallagher (1966), Tay & Biagi (1968), Bennett (1975a,c) and Bennett & Threadgold (1975) on F. hepatica. Although no such study has so far been made on F. gigantica there appears no possibility that at it's ultrastructural level would there be any specific deviation from the generalized pattern of F. hepatica.

The liver-flukes were generally believed to feed on the bile contents of the liver and gall bladder and the much ramified caeca and multiple branched digestive system does provide sufficient testimony in this regard but, based on the findings of Stephenson (1947b), Gresson & Threadgold (1959), and his own, Dawes (1962,& 1963 a,c) furnished conclusive evidence that F. hepatica feeds on the hepatic tissue of it's host as well.

Among other notable contributions on various trematode systems relating liver-flukes are those of Lang (1880), Sommer (1880), and Bettendorf (1897) on it's generalized pattern of nervous system, Bugge (1929), Kawana (1940) and Pantelouris & Threadgold (1963) on the excretory system, and Shyamasundari & Rao (1975) on the neurosecretory cells of F. hepatica and F. gigantica. These are substantiated by the histochemical studies of Weinland and von Brand (1926), Monne (1959), von Brand & Mercado (1961), Bjorkman et al. (1963), Pantelousis (1964), Threadgold and Gallagher (1966) and Halton (1967 a,b). However, no such studies have been made on F. gigantica.

The reproductive system, particularly the Mehlis' gland complex, cytology of gonads, spermatogenesis, oogenesis, germ cell cycle, and mechanism of egg shell formation have been subjected to elaborate studies by various investigators, notable among these are the contributions of Gresson (1957), and Yosufzai (1952a & 1953a) on germ cell cycles, spermatogenesis and oogenesis in F. hepatica and of Smyth (1951), Yosufzai, (1953b) and Rao (1959a,b & 1960) on egg shell formation. However, the only study on this aspect in F. gigantica is that of Rao & Madhavi (1962) on the Mehlis' gland complex.

I. THE TEGUMENT

The generalized tegument of trematodes comprises an outer granular protoplasmic cuticle, delimited by an outer and a basal plasma membrane. The 'cuticle' bears spines projecting out of it still enclosed within the two plasma membranes. The cuticle is followed by an amorphous basement layer to which are attached peripheral muscles. The protoplasmic cuticle is in continuity with the subcuticular cells lying beneath the peripheral muscles in close association with parenchyma cells.

Regarding the structure, composition and origin of the trematode cuticle, several contributions have been made by earlier investigators. Hyman (1951), has summarised the comparative morphology and also discussed the different homologies, Alvarado (1951) has extensively discussed various theories, but could not differentiate various regions of the body wall by using light microscopy and the histological techniques. Much information was added by later workers viz., Pantelouris and Gresson (1960). Prior to 1959 the cuticle was considered as an inert layer, homologous to the invertebrate chitin, but with the advent of electron microscopy it has since been regarded as a protoplasmic and metabolically active layer. Though the first study in this regard was made by Senft (1959) and Senft *et al.* (1961) on *Schistosoma mansoni*,

it was soon followed by outstanding contributions by Threadgold (1963a,b), Bjorkman and Thorsell (1964), and Tay and Biagi (1968), on Fasciola hepatica, and relatively more recently by Bennett (1975a,c) and by Bennett and Threadgold (1973 & 1975) who have studied its surface features through scanning electron microscopy.

The composition of different cuticular layers of trematodes have been extensively substantiated histochemically by many workers during the last three decades; notable contributions among these are those of Yamao and Saito (1952), Berthier (1954), Monné (1959), Lal and Shrivastava (1960), von Brand and Mercado (1961), Pantelouris and Hale (1962), Becejac and Kravavica (1964), Pantelouris (1964), and Halton (1967a,b) and comparatively recently by Lee (1966 & 1972), Hockley (1973) and Lyons (1977). Most of these studies were carried on F. hepatica.

The tegument of Fasciola gigantica essentially comprises an outer cuticle, about 20 µm thick, delimited by a uniform strech of an ultra-thin outer layer and a basal plasma membrane (Pl. II, 1). This is a uniform granular layer which includes vertical rows of granules. The outer most surface of the cuticle, including the plasma membrane shows plateaux and valleys in transverse as well as in vertical sections (Pl. II, 1). The valleys, as appearing in vertical sections,

are deeper than those in transverse sections and appear to out number the former. In frontal sections through the outer surface of the cuticle, two types of folds appear : (i) transverse valleys forming rectangular ridges and plateux around the centrally projecting spines, and (ii) longitudinal corrugations, which are fine, and run parallel over the transverse ridges and valleys (Pl. XVI, 6). These plateux, valleys and longitudinal corrugations more frequently occur beyond the acetabulum and are either sparce or ill defined transverse valleys on the anterior region. The cuticle appeared glycogen free but PAS positive granules, non-liable to diastase digestion were abundant in the upper part of the cuticle though scant in the general tegument. The upper most part seemed to have a muco-polysaccharide coat (Pl. XVII, 2). The cuticle, including the epicuticle is highly protenaceous. This protein is slightly basic (Pl. XXVII, 2), while the epicuticle appeared negative to basic proteins. This is also slightly sudanophilic but the epicuticle shows negative result (Pl. XVII, 6). No bound lipid is present in the general cuticle. The RNA is also observed present in the cuticle.

The cuticle also bears hyaline spines. These are spatulate, appearing generally broader at it's base and sharper at the tip in vertical profile (Pl. II, 1; XVII , 5). Major portion of the spine is embedded within the cuticle and

generally 1/4 to 1/3 projects from the cuticular surface, which too appears to be covered by the outer cuticular plasma membrane (Pl. II, 1). The spine's base rests in a shallow depression on the basement layer and does not appear to indent itself. The size of the spines markedly vary in different body regions, measuring from 15 x 30 to 40 x 60 μm on the anterior region, 40 x 65 to 60 x 70 μm near the genital atrium, 50 x 80 to 60 x 100 μm below the acetabulum; and 25 x 30 to 30 x 45 μm near the posterior end (Pl. II, 2; III, 1-3). These spines are directed posteriorly (Pl. III, 4) from the hinder acetabular margin and exhibit a definite spatial quincunxial pattern (Pl. XVII, 1). However, they vary inversely to their size in their number per square mm area in different regions. They present about 22/mm^2 on the anterior region, 4/mm^2 below the acetabulum and throughout the middle region; and 10/mm^2 on the posterior region. The outer margin of the spines is not generally regular but is finely serrated (Pl. II, 2; III, 1-3). Histochemically these spines are found highly protenaceous, showing strongly basic proteins. They also stain deep red with Azan dye (Pl. XVII, 5) and appear glycogen free (Pl. XVI, 1; XVIII, 1,2).

The cuticle is followed by a uniform basement layer, about 5 um in thickness. This layer appears to have formed distinct arches intercalated with the base of the cuticle (Pl. II, 1). The basement layer stains blue with Azanophilic

dyes (Pl. XVII, 5). It is glycogen free, moderately protenaceous (Pl. XVI, 1; XVIII, 1-3),but rich in bound lipids (Pl. XIX, 2), and reticulin. This layer is followed by peripheral musculature.

Beneath the peripheral musculature are found the flask shaped subcuticular cells in clusters, each measuring about 8-10 um with a centrally placed nucleus (Pl. II, 1). The nucleus contains a large, distinct, usually centrally located nucleolus. In addition to it, certain haematinophilic fragments also occur in the nucleus. Fine processes proceed from these cells, branching distally, extending upto the 'cuticle', traversing through the basement layer and basal plasma membrane. These processes pass through the lacunae left by the cris-cross arrangement of transverse and longitudinal muscles (Pl. XVII, 3). In transverse sections these processes are observed passing through intercellular spaces of longitudinal muscles and are partly covered by circular muscles, while a reverse condition is observed in vertical sections. These may be regarded as tegumental cells Type-I, as were reported by Threadgold (1967) in F. hepatica. In these clusters other types of cells are present which occur closely apposed to the above cells, less frequently, generally 1 to 2 in a cluster. The number, however, is not fixed. These cells are larger than the Type-I cells mentioned. The

single cell measures about 15 µm, with a distinct large oval or round nucleus, measuring 8 µm in diameter. The central or acentric nucleolus is fairly large of all cells (Pl. II, 3, 4). The cytoplasm is comparatively less granular than in type-I cells and the nucleus shows sparce chromatin deposits. The most obvious feature of these cells is the occurrence of large number of small non-glycogen PAS positive granules. These inclusions are randomly dispersed throughout the cytoplasm of the cell type-II. These organoids are probably analogous to the disk-like membrane-bound secretory bodies, as described in the tegumental cells of F. hepatica (Bjorkmann & Thorsell, 1963; and Threadgold, 1967). The protoplasmic projections of these cells, unlike those of Type-I, project upward, probably joining the syncytial tegument (Pl. II, 3, 4).

In these clusters, certain cells have been observed juxtaposed to the muscle fibres. In F. hepatica such cells have been designated as myoblasts (Threadgold, 1963a and Pantelouris, 1965). The cytoplasm of the general tegumental cells and the myoblasts, lying within the same cluster, reflect a differential picture with Heidenhain's Azan, as the cuticular cells show highly granular cytoplasm while less granular appearance with frequent presence of vacoules in myoblasts (Pl. II, 1). The cuticular cells do not possess glycogen, but certain PAS positive granules are frequently

present in these cells, though small deposit of glycogen has been observed in myoblasts. Lipid droplets are usually found in the sub-cuticular cells but rarely, if at all, in the myoblasts.

Interspersed among these cells are found, in good number, large distinct cells which respond effectively, with Bargmann's haematoxylin, and are therefore inferred as neurosecretory cells. Such cells have also been reported in other trematodes. A detailed description of these cells, as found in F. gigantica is given elsewhere (Chapter V).

Details of various histochemical tests performed on the tegument have been furnished in Table III.

Alvarado (1951) and Pantelouris and Gresson (1960) have also reported the presence of distinct nuclei in the cuticular region of F. hepatica, though such nuclei could not be observed in F. gigantica, Threadgold (1963a,b) has also denied the presence of such nuclei in F. hepatica. He has, instead, reported the occurrence of a mucopolysaccharide coat which has also been noted by the present author in F. gigantica. Bjorkman et al. (1963) have indicated this coat as a derivative of mucoproteins in F. hepatica whereas recent workers (Hockley, 1973; Stein & Lumsden, 1973) have considered it as an acid-containing glycoprotein possibly of antigenic nature. This muco-polysaccharide coat has now been

termed as the Glyco-Calyx.

The most notable superficial cuticular modifications are the longitudinal corrugations. The transverse valleys have also been observed by Threadgold (1963a,b) and Bennett (1975a,c) in F. hepatica. In F. gigantica these corrugations are abundant in the post-acetabular region. Other main functional significance appears to be the furnishing to maximum surface and contact area, as also flexibility for the movement of the spines.

The spines seem to act as secondary organs for attachment (Bennett, 1975c) in F. hepatica. Dawes (1963c) has attributed to them the functional adaptation for migratory feeding in the bile-duct, the sinusoids and the liver parenchyma. Considering their shape and direction (spatulate and posteriad), if there is a mere chance of their movement, they can exhibit it only in to and fro direction. It is assumed that they prevent the backward drag of the worm against the fluid current while the suckers are relaxed.

The presence of cholinesterase in the tegument of F. gigantica may be attributed to the frequent innervation under the subcuticular region. Halton (1967b) has reported marked cholinesterase activity in F. hepatica. This enzyme

has been distinctly observed to have been localized in the subcuticular region of F. gigantica. The neurofibrils containing acetylcholinesterase have been found to be distinctly and positively stained by Indoxyl acetate and acetylthiocholine iodide method (Pl. XXX, 3-6) which furnished adequate evidence of the presence of this enzyme.

Regarding the origin of cuticular layer in trematodes the views of Hyman (1951) appear to be more tenable. Accordingly "the cuticle is the outer layer of an insunk epidermis, the cells and nuclei of which have sunk beneath the subcuticular musculature". Secretory functions have also been attributed to the sub-tegumental cells (Bjorkman & Thorsell, 1964 and Threadgold, 1963a,b & 1967), and similar conclusions have been arrived at, through histochemical investigations relating to the present study.

TABLE - III

Results of various histochemical tests performed on the tegument of Fasciola gigantica.

Tests performed	Spine	Epicuticle	Cuticle	Basement layer	Peripheral muscles	Hypodermal cells	Myoblasts
PAS	-	++	-	++	++	-	+
PAS, after diastase digestion	-	++	-	+	-	-	-
Best's Carmine	-	-	-	-	++	-	+
Best's Carmine after diastase digestion	-	-	-	-	-	-	-
Mercury bromophenol blue	+++	+++	+++	++	+++	++	++
Acid Solochrome Cyanine	+++	-	+	-	++	++	++
Alcian blue	-	++	-	-	-	-	-
Pyronin Y & Methyl green	-	++ Pink	++ Pink	-	-	++ Pink	+/- Pink
Sudan black B	-	-	++	-	+	+	-
Acetone Sudan black B	-	+	-	+++	+	-	-
Indoxyl acetate	-	-	++	-	-	-	-
Indoxyl acetate after Eserine	-	-	-	-	-	-	-
Acetylthiocholine iodide	-	-	++	-	-	-	-

+++ = intensely stained; ++ = moderately stained; + = slightly stained; - = no stain +/- = not known.

II. THE PARENCHYMA

The parenchyma occupies major portion of platyhelminth interior. It essentially comprises a network of loosely spaced connective tissue with large, closely apposed cells appearing polygonal in sections. The anastomoses of these cellular processes form a reticulate net-work all over. Dispersed within this connective tissue, are found certain specialized large cells, sparcely distributed throughout the body. These have been described in detail in Chapter V.

There are frequently present large spaces of various size within the parenchyma cells. In between the adjoining parenchyma cells, distinct spaces have been reported. The nature of the spaces has been subject to much speculation. Some investigators considered them intracellular and some as extracellular (Wisniewski, 1930). However, the latter view, mainly based on the descriptions of Ortner-Schoenbach (1913) and Prenant (1922) is widely upheld, and has further been supported by Alvarado (1951), and Bjorkman and Thorsell (1962) who have substantiated this view through ultra-structural investigations. The findings of Threadgold and Gallagher (1966) and Gallagher and Threadgold (1967) on F. hepatica lend further credence to this hypothesis.

In Fasciola gigantica, the parenchyma is essentially a compact network inbetween the various systems and structures

throughout the body, even inbetween the redial muscle bundles of the suckers and the pharynx. It comprises closely apposed cells which considerably vary in shape and size. Some are small and round, whereas others are elongated, measuring approximately 25-35 µm in cross sections (Pl. IV, 1). The shape and size of the nuclei also vary considerably (Pl. IV, 4), measuring from 5 - 6 µm, being spherical, oval, or reniform, with a small spherical or amoeboid median nucleolus. The nucleus essentially lies towards one side of the cell. The cytoplasm of the parenchyma cells appears granular and vacolated (Pl. IV, 1). The vacoules do not appear so frequently and large in Best's carmine and PAS stained preparations (Pl. XVI, 3; XX, 4 and XXVII, 5). The majority of the cell inclusions within these vacuoles are certainly glycogen deposition of various macromolecular size, sometimes appearing exceptionally large and rounded in shape (Pl. IV, 1). This distribution of glycogen appears intense in both the anterior as well as in the posterior regions. The cytoplasm is moderately protenaceous (Pl. XXIV, 2). This protein moiety is not basic, and the amount of bound proteins invariably appear scanty (Pl. XXVII, 2). Fat droplets are sparcely found in these cells (Pl. XXV, 4), while some bound lipid is present (Pl. XXIV, 3). The general cytoplasm of paranchyma cells do reveal the presence of RNA (Pl. XVII, 4).

The cell boundaries are well defined in F. gigantica,

measuring generally about 2 μm in thickness. Sometimes the two adjacent plasma membranes are separated by a much thicker intercellular material (Pl. IV, 1-3), which varies greatly in thickness at different places. The intercellular material shows a hyaline appearance including non-homogenous, reticulin. There also appears ample quantity of bound lipids within this intercellular material and also sometimes fine granules of PAS positive non-glycogen material.

The parenchyma cells are in close association with the intestinal crura, the reproductive system, body wall, and the excretory system. The cells, surrounding the intestinal caeca seem to possess short pseudopodia-like projections towards the base of the intestinal epithelial cells passing through the circular muscle bundles, interstitial material, and probably reaching the basement layer, which is not so clear under light microscopy (Pl. IV, 2, 3). The membranes of the parenchyma cells and intestinal epithelial cells appear comparatively thin at this point.

The close association of the paranchymatous cells with the tegument seems similar to that of the intestinal caeca except the pseudopodial projections which do not reach up to the cuticular base.

Details of various histochemical tests performed on the parenchyma have been furnished in table IV.

The parenchyma of F. gigantica is essentially similar to that of F. hepatica. Threadgold and Gallagher (1966) considered intercellular material as the interstitial material in F. hepatica. The exact chemical and functional nature of this material is still unknown, nevertheless the result of present study enables the writer to regard it as reticulin, which has also been reported in F. hepatica (Prenant, 1922 and Alvarado, 1951). Either these are secretory products or of some other origin. This is still unknown although, Alvarado (1951) has suggested it as a secretory product of the mesenchyme. The present author is of the view that these materials; appearing within the intercellular spaces are usually under the inter-cellular transit.

The vacuoles are presumably sites of glycogen deposits, which are either lost as a routine fixation artifact or they might remain ill-defined or even unstained with routine stains. Pantelouris (1964) also postulated similar idea about such vacoules.

As present study reveals, the parenchyma beyond a packing system is a major glycogen storage organ, similar to the one in F. hepatica (von Brand & Mercado, 1961), and is probably metabolically and physiologically active, as was suggested by Halton (1967a) for F. hepatica.

The presence of cytoplasmic RNA also suggests the presence of granular endoplasmic reticulum in these cells.

The closest association of this system to all other systems, particularly such as the intestinal crura and tegument also suggests the probable distributory performance of this tissue to various systems, and this aspect is a compensation to the absence of a circulatory system.

The presence of fat is mostly in the form of bound lipids and this moiety is probably derived through the sterols present in the host blood, and is converted to bound lipids metabolically within the parenchyma. This confirms the fact that it also feeds on host blood in the sinusoids as was suggested by Dawes (1963a, c) for F. hepatica.

TABLE - IV

Results of various histochemical tests performed on the parenchyma of *Fasciola gigantica*

Test performed	Parenchyma		
	Ant.region	Post-region	Intercellular material
PAS	+++	+++	++
PAS, after diastase digestion	-	-	+
Best's Carmine	+++	+++	++
Best's Carine, after diastase digestion	-	-	-
Mercury bromophenol blue	++	++	+++
Acid Solochrome cyanine	-	-	+
Alcian blue	-	-	-
Pyronin Y & Methyl green	+ Pink*	+ Pink*	+ Pink
Sudan black B	++	++	-
Acetone Sudan black B	+	+	-
Silver impregnation	-	-	+
Indoxylacetate	-	-	-
Indoxyl acetate, after Eserine	-	-	-
Acetylthiocholine iodide	-	-	-

* Confined to perinuclear region

+++ = intensely stained; ++ = moderately stained; + = Slightly stained; - = no stain.

III. THE DIGESTIVE SYSTEM

The generalized pattern of digestive system in the genus Fasciola comprises a subterminal oral sucker, followed by a well developed muscular pharynx, a short oesophagus, blind intestinal caeca reaching the posterior extremity, provided with dendritic branches on either side. The intestine is throughout lined by (Epithelium) the gastroderm.

Early works on the digestive system of Fasciola spp. were those by Sommer (1880) and Leuckart (1881) on F. hepatica. Many investigators have studied or discussed the functional morphology of F. hepatica, such as Müller (1923), Stephenson (1947c), Gresson and Threadgold (1959) and Dawes (1962). Recently the function of the gastrodermis of F. hepatica has been substantiated by histochemical and/or ultrastructural studies by many workers. Notable among them are those of Gresson and Threadgold (1959) on electron microscopic studies of the caecalcells of F. hepatica, Thorsell and Bjorkman (1965) on the absorption and secretion in the alimentary tract, Gallagher and Threadgold (1967) on the interrelationship between parenchyma and gastrodermal cells, Halton (1967a,b) on the localization of enzymes in the intestinal epithelium, Threadgold (1968) on the ultrastructural localization of phosphatase, Bennett (1975b) on the development of the caecal epithelium during migration in the host, and of Rohinson and

Threadgold (1975) on the fine structure of gut epithelium. All these studies were on F. hepatica.

The digestive system of Fasciola gigantica essentially comprises an anteriorly situated mouth, encircled by a subterminal oral sucker, measuring about 1.0 mm in diameter, forming a funnel-like cavity, the lumen of which passes through the doliiform pharynx. The pharynx is divisible into a prepharynx and the main pharynx (Pl. VI, 2, X IX, 1). This is followed by a short, transverse oesophagus, on either side posterior to the pharynx. In this case the intestine does not directly fork, but it is the oesophagus which lends into the corresponding intestinal caecum on either side, ensuing from an inverted Y-shaped oesophagus. The lumen of the oesophagus is lined by the cuticle, continued through the pharyngeal lumen (Pl VI, 2). In some cases the oesophagus appears attenuated instead of being inverted Y-shape, depending on the up/downward movements of the pharynx. The structural details and muscular arrangement of the oral sucker and the pharynx have been described elsewhere (Chapter IV). The results of histochemical tests on oesophageal lining shows similar responses as in general tegument (Table V).

Both the intestinal caeca are blind posteriorly and are ramified into numerous long, dendritic outer branches, and fewer shorter inner branches. The inner branches appear beyond the acetabulum, and traverse antero-posteriorly. These

are wider than the outer ones. The outer branches further divide generally, upto tertiary level towards the margins (Pl. I, 2).

The gastrodermis

The gastrodermis commences after the either end of the oesophagus (Pl. VI, 2), and comprises single celled columnar epithelium, resting on a basement layer (Pl. V, 1). There are two morphologically distinct types of gastrodermal cells; (i) the short forms measuring 8 x 15 µm, (ii) the large forms each being 8 x 35 µm in size. No specially localized area is specific for the distribution of these two morphological types. Empty gut generally shows columnar (long) types while gorged caeca exhibit cuboidal cells. In both types of gastrodermal cells, the nuclei are generally located near the basal region, each nucleus being 5 µmin diameter and containing a small, round, central nucleolus. The apex of the long columnar cells is provided with digitate fringes, which reflect a brush border-like appearance (Pl. V, 4).

The cytoplasm is dense granular. The basement layer appears closely situated with plasma membrane of the gastrodermal cells and also the underlying parenchyma cells (Pl. V, 4). The two types of aforesaid cells do not differ histochemically from each other. As both of these appear

to have protenaceous nature. These contain basic proteins (Pl. XXVII, 2). Fairly good amount of RNA seems to be present in the epithelial cells indicated by Pyronin Y and methylgreen test. The fat is also localized in these cells with more abundance in the basal regions (Pl. XXVII, 6). There is no bound lipid in these cells (Pl. XIX, 2). Large amount of non specific esterases are also present in the gastrodermal cells.

The basement layer shows similar histochemical nature as previously described in the tegument (Table II). On the outer side, next to the basement membrane lie the circular muscles or periintestinal muscles (Pl. V, 1 & VIII, 2) on which, at places, the dorsoventral muscles are attached (Pl. VIII 2,4). Results of the histochemical studies on the digestive system have been furnished in Table V.

Till now the oesophagus of the genus Fasciola was described as a short straight tube but the present study has revealed that it is a bifurcated, inverted Y-shaped structure on the basis of it's histological details which are quite different from the adjoining intestinal caeca.

The feature of the intestinal caeca seems not to be functionally so significant but are in accordance with the display of other organ systems particularly in the middle posterior region where lies the excretory vesicle. Thus

the intestinal caeca can not ramify to a finer level on the inner side. The outer branches repeatedly divide ultimately assuming an arboreal pattern. Presence of the tall cells in the empty gut and short cuboidal cells in distended one is a condition similar to the one in F. hepatica as was described by Gresson and Threadgold (1959) who interpreted these two types of cells as either inter-changable phases, depending on the presence or absence of food and suggested them as absorptive and secretory phases or some cells are absorptive throughout and change their shape and size when required. Dawes (1962) suggested an apocrine mode of secretion and presumed the tall cell processes for releasing their secretion into the lumen of the gut. According to Robinson and Threadgold (1975) there is only one cell type in the digestive caeca of adult F. hepatica, which alternate between absorptive and secretory phases. This idea seems to be more plausible because there is no necessity of any cell being throughout absorptive, while the intestine is without food when the gut contents are vomitted out. During this intervening period the absorptive cells may prepare to perform as a secretory cell when the food again comes in contact with these cells. While the secretory function is over, the same cell may possibly perform an absorptive function. Thus a secretory and absorptive cycle seems to be operating within single cell. The cell processes of tall gastrodermal cells

are perhaps similar as were described as microvilli-like structures in the caecal cells of certain digeneans (Wootton & Sogandares-Bernal, 1963 & Halton, 1966).

The presence of lipids and acid mucopolysaccharides in the gut epithelial cells suggests it's permeable and protective functions. The two moities have also been reported in such cells (Nacheva, 1977) in F. hepatica, which substantiate the aforesaid function of the gut epithelium. Beyond the absorption the gastrodermal cells probably also perform other metabolic functions such as protein synthesis. Substantial amount of RNA and Alkaline phosphatase in these cells also supports this idea. The same view was upheld by Hanna (1975) for the gut epithelial cells of F. hepatica. The presence of RNA may presumably be correlated with the presence of granular endoplasmic reticulum in the gut epithelial cells of F. gigantica and this will be worth an investigation.

The fluke gulps large quantities of bile, and perhaps blood and also perhaps feeds on host (liver parenchyma) cells through the acetabular - pharyngeal coordination and after the digestion, the gut contents are probably vomitted in the habitat. Contraction of dorso-ventral muscles and also linear contraction seems to facilitate this process which has been observed in the in vitro conditions where in

washed and debric-cleaned F. gigantica specimens, were kept in Hedon-fleig's solution.

TABLE - V

Results of various histochemical tests performed on the digestive system of Fasciola gigantica

Tests performed	Gastrodermal cells (long)		Gastrodermal cells (short)	
	Basal region	Apical region	Basal region	Apical region
PAS	-	+	-	-
PAS, after diastase digestion	-	+	-	-
Best's Carmine	-	-	-	-
Best's Carmine, after diastase digestion	-	-	-	-
Mercury bromophenol blue	+++	++	+++	++
Acid Solochrome cyanine	+++	+++	+++	+++
Alcian blue	-	-	-	-
Pyronin Y & Methyl green	+++ Pink	++ Pink	+++ Pink	+++ Pink
Sudan black B	+	+++	++	+++
Acetone Sudan black B	-	-	-	-
Silver impregnation	-	-	-	-
Indoxyl acetate	++	++	++	++
Indoxyl acetate after Eserine	++	++	++	++
Acetylthiocholine iodide	-	-	-	-

+++ = intensely stained; ++ = moderately stained;

+ = slightly stained;- = no stain.

IV. MYOARCHITECTURE

Musculature in the trematodes is one of the most developed systems, sustaining the body shape and movements. This system comprises muscle fibres arranged in bundles and invested in a network of reticulin (Alvarado, 1951), containing nuclei in the muscle fibres, and the myoblasts attached with the former.

Although fairly well developed in the Trematodes, this system has not been extensively worked out, except preliminary studies by Bettendorf (1897), Alvarado (1951) and Pantelouris (1965).

In F. gigantica, the lay-out of the somatic muscles is as follows:

Peripheral Musculature

Beneath the Hypoderm there is a layer of transverse muscles, comprising delicate fibres, attached to the cuticular basement layer, covering about 10 μm in thickness. Following this layer is the longitudinal muscle layer, about 15 μm thick. The fibres of both muscle layers are arranged in definite bundles (Pl. II, 1). These two types of muscle bundles criss-cross each other forming distinct lacunae (Pl. XVI, 2; XVII, 3).

This peripheral musculature occurs throughout the body as a muscular sheath.

These muscle bundles are enclosed by an interstitial material. The nuclei are also present in the muscle fibres, more frequent in the longitudinal than the transverse muscles, each measuring about 5 μm. The myoblasts exist in many possible shapes and size. The detailed structure of these myoblasts has already been given in Chapter I. There may be present one myoblast, attached to single fibre or single myoblast could be attached to many fibres through cytoplasmic processes (Pl. VIII, 1-5; XVI, 4). Delicate neurofibrils arising from nerve cells extend upto the muscles where these fibres have been observed to end in minute nerve endings at places.

Anterior diagonal musculature

The diagonal muscles form an arch-like pattern between the oral end and the anterior margin of the acetabulum (Pl. VI, 3). These are present both on the dorsal as well as the ventral sides in the same region and are arranged in definite bundles in a cris-cross pattern (Pl. VI, 1). Nuclei of the muscle fibres, myoblasts, and nerve endings are also found in these bundles (Pl. VIII, 9-13). In transverse sections these muscle bundles appear in an arch; the two ends

of which do not attach at the exact lateral points, but slightly away from the longitudinal muscles (Pl. VI, 3). The myoblasts related to these muscles are most pronounced. They are found even in the formative stages with prolongation of muscle fibres (Pl. VII, 5; XXVI, 1).

The dorsoventral musculature

Distinct muscle bundles commencing from dorsal and ventral peripheral longitudinal muscles traversing dorsally and ventrally (Pl. VI, 3-5) are present throughout the body. Some of these are fairly well developed and traverse from dorsal to the ventral side, mainly along the lateral margins, but some of them end midway and are attached at the intestinal crura (Pl. VII, 4). The nuclei are most frequent and prominent in these muscle fibres and the large-sized myoblasts are also attached to them (Pl. VIII, 9-12). These muscles are also adequately innervated which is evidenced by the presence of numerous nerve-endings terminating thereat (Pl. VIII, 13; XVI, 3).

Musculature of the Acetabulum

The acetabulum, measuring 1.5 mm in diameter, is a cup-shaped structure. Its cavity is provided with the non-spinose cuticle and dorsally it is delimited by the basement membrane (Pl. XX, 2). The outer most muscular layer comprises

meridional fibres, having nuclear profile like that of the peripheral transverse muscles (Pl. VIII, 1). These fibres are attached over the basement layer. There are characteristic radial muscles attached to the meridional fibres showing striated pattern. The marginal radial muscles are more compact and relatively thicker bundles than the central ones (Pl. VIII, 1). The spaces in between the radial muscles is packed with parenchyma. Myoblasts and nerve endings are observed in this region (Pl. VIII, 6-8, 14-16). In between the radial muscle bundles,particularly on marginal sides and outer and inner periphery run the circular muscle fibres. These circular muscles are compactly arranged under the edges of the sucker, forming a sphincter. In addition to these, the dorso-ventral muscles are also attached to the basement layer of dorsal side of the acetabulum from all sides (Pl. VII, 1). At places, particularly on the lateral side, muscles from dorsal side cross those of the ventral side (Pl. VI, 5).

Musculature of the oral sucker and the pharynx

In F. gigantica, the oral sucker is a funnel-shaped structure with a slightly basal extention on the ventral side (Pl. IX, 8-10). The funnel cavity is lined by the cuticle, measuring about 10 um in thickness. The inner limiting outline lacks the cuticle, as found in the acetabulum but the uniform strech of basement layer extends from the

Muscles (Pl. VI, 2), which probably facilitate the upward and downward movements of the entire pharynx.

Periintestinal muscles

The intestinal crura are surrounded by delicate circular muscles which are attached to the basement layer underneath the intestinal epithelium. To these circular muscles are attached dorsoventral muscles in all directions (Pl. VII, 2). Nuclei and myoblasts are also found in and along these muscles.

The cytological and histochemical details of these myoblasts have been discussed elsewhere (Chapter I), and the histochemical nature of all types of muscle fibres are similar to those described for peripheral musculature in Chapter I, Table III.

The transverse and longitudinal peripheral muscles are responsible for their complex body movements. Contrary to the view of Pantelouris (1965), nuclei are found in the peripheral musculature in F. gigantica, in the ascending frequency of the sequence of muscle's position of the worm's body. The nuclei are found in various shape and size in different muscles. With this fact the present author intend to draw a hypothesis that the frequency of nuclear presence in the muscles is probably dependent on the age of the muscle. In other words, the sequence of development of various body muscles can be determined with the help of number and shape of the nuclei.

adjacent body wall (Pl. XVIII, 6; XX, 4). Centrally there is an opening. The outer most layer measures about 10 µm thick and passes through the lower central opening of the sucker. The radial muscles are arranged in a regular manner. On the inner and outer margins, inbetween the radial muscles run the circular muscles, specially on the marginal sides, forming a sphincter. The opening from the middle of the oral sucker leads into an oral muscular pharynx, divisible into an upper prepharynx and the lower 'pharynx'. This division is superficial, internally there is no structure to separate the two parts. There appears a circular muscular constriction in between the prepharynx and the basal opening of the oral sucker, the interspaces being occupied by the parenchyma internally, and covered with the continous cuticle of the sucker cavity externally which also extends into the lumen of the pharynx. This zone is fairly innervated by a neural plexus (Pl. VI, 2). The outer muscle of the pharynx is a continuous circular sheath and there radial muscles are also present. The spaces are packed with parenchyma on both the margins of pharynx. Next to the circular muscles run the longitudinal muscles. The outer circular muscle layer is dorsal to the basement layer and at the region of oesophageal junction they form a sphincter. In addition, two stout lateral muscle bands run longitudinally starting from the base of the pharynx and are attached to the lower base of the oral sucker. These are probably protractor and retractor

In case this inference is tenable, the sequence of development of different muscles should be as follows: the outer transverse muscles and the meridional muscles of the suckers as well as the outer circular muscles of pharynx seem to develop first, and the anterior diagonal musculature later. This is evidenced by the cytological details of these diagonal muscles which show many proliferating fibres with large amount of perinuclear cytoplasm.

Contrary to Alvarado (1951), the circular muscles arranged in the intestinal crura seem to perform peristaltic movements, while the dorsoventral muscles attached to these circular muscle may be responsible for dilation and contraction of the intestinal lumen. There might be the possibility that the dorsoventral muscle bundles may create alternate contractile and extensile activities thereby resulting peristaltic movements in the intestinal crura.

V. THE NERVOUS SYSTEM AND THE NEUROSECRETORY CELLS

The nervous system

The generalized nervous system of trematodes consists of a pair of anterior ganglia situated near the adpharyngeal region which may be connected with a commissure and a variable number of longitudinal nerves, extending anteriorly as well as posteriorly. These longitudinal main nerve trunks may be joined in some cases by transverse connectives, and many lateral branches may arise innervating various systems of the worm.

Although elucidation of the nervous system in the Platyhelminthes has been quite problematic, it did attract the attention of earlier investigators in the 19th century. Outstanding contributions in this regard are those of Lang (1880), Sommer (1880), Bettendorf (1897), and Havet (1900), who worked chiefly on Fasciola hepatica. The contribution of Bettendorf is still considered as one of the best descriptions of this system. More recently the ultrastructure of the nervous system in the cercarial stage of F. hepatica was studied by Dixon and Mercer (1965). These workers and their contemporaries, Ude (1962), and Rohde (1965) also suggested a neurosecretory system in the digenetic trematodes. Lately,

Gresson and Threadgold (1964), and more recently Shyamasundari and Rao (1975) reported the occurrence of neurosecretory cells in Fasciola hepatica and F. gigantica.

The nervous system of F. gigantica comprises a pair of large anterior ganglia, each measuring about 0.2 x 0.25 mm and situated on the lateral sides in adpharyngeal region (Pl. IX, 2). These ganglia are connected with each other through a broad arched dorsal commissure (Pl. IX, 1,2,10). In these ganglia are found some PAS positive substances. It is also moderately protenaceous and the fat moiety is highest in all the neural structures in this region (Pl. IX, 4). Cholinesterase is also present actively in the ganglia (Pl. XXIX, 1,6).

From these ganglia arise 6 pairs of nerves, 3 of which run anteriorly and remaining, posteriorly. Out of each three pairs, one is lateral, one dorsal and the third, ventral (Pl. IX, 1,2). The anterior 3 pairs traverse along the oral sucker (Pl. IX, 2,4-10) and the posteriads reach the posterior extremity of the worm (Pl. IX, 1). There is a separate nerve innervating the either side of the prepharyngeal region and one short pair extending upto the posterior ends of doliiform pharynx (Pl. IX, 2; XIX 3,4). Among the posterior three pairs of longitudinal nerves, one pair runs laterally, reaching upto the posterior extremity but does not meet each other. Out of the remaining two pairs, the outer one is

ventral and in relatively thicker than the main nerve trunks which run parallel to the body axis. The inner one is dorsal in position. The dorsal nerves innervate mainly the uterus, the genital atrium and the ovary. The post-ventral nerves at the region of ventral sucker give rise to corresponding inner branches which further ramify and ultimately innervate the acetabulum. At the level of Mehlis' gland complex, from each ventral nerve arises an inner transverse branch and each enters the either side of the complex to form two distinct nerve plexuses (Pl. IX, 3). At the same level outer transverse branches also run parallel to the transverse vitelline duct of the corresponding side (Pl. IX, 3). In the field below the posterior testis, the ventral and the dorsal pairs anastomose with the inner finer branches, while the outer transverse connectives from both pairs meet the lateral nerves (Pl. IX, 1). The transverse branches of the ventral nerves are well developed than the dorsal ones. The terminal ends of the ventral nerves meet each other to form a peri-vesicular ring around the excretory bladder (Pl. IX, 1). From the two lateral junctions of this ring fine nerves arise to innervate the excretory sphincter region (Pl. IX, 1). This region also shows an intense activity of cholinesterase (Pl. XXIX, 3,4).

The main nerve trunks and thick branches show the presence of certain non-glycogen PAS positive materials

along them (Pl. XXVI, 5). These are also moderately protenaceous (Pl. XXV, 3) and show a fat moeity(Pl. XVI, 5; XXV, 4). The cholinesterase is localized along the entire nervous system including the main nerve trunks as well as finer nerve branches (Pl. XXVIII, 1; XXIX, 1,6; XXX, 3-6). Out of the anterior 3 pairs of nerves, the ventral pair appears more distinct than the other two pairs.

The nervous system of F. gigantica can be localized by using the histochemical technique of localization of the acetylcholinesterase in toto with considerable success. This technique has variously been adopted to workout the nervous system and neuroanatomy in various helminth parasites with a remarkable success (Schardein & Waitz, 1965; Ramisz, 1967; Shield, 1969; Wilson & Schiller, 1969 and LeFlore & Smith, 1976). The enzyme has already been reported to be amply present in F. hepatica (Bacq & Oury, 1937; Chance & Mansour, 1953 and Sekardi & Ehrlich, 1962).

There are 6 distinct pairs of longitudinal nerves in F. gigantica and the same number has been reported in F. hepatica (Plantelouris, 1965), whereas, Bettendorf (1897) described only 4 pairs in the same fluke.

The neurosecretory cells

The neurosecretory cells in the invertebrates, particularly in the Digenea have been demonstrated by many

workers using chrome haematoxylin-phloxine technique. In Fasciola hepatica these cells were first reported by Gresson & Threadgold (1964) and subsequently by Grasso (1967 a,b & 1972) and Shyamasundari & Rao (1975) furnished preliminary histochemical account of these cells in Fasciola hepatica and also in F. gigantica, and differentiated these cells into two kinds viz., type 'A' and type 'B'.

In context with the present studies on F. gigantica, neurosecretory cells have been observed in the brain as well as in certain other regions. Structurally these are of two types; which can be differentiated on structural basis to certain extent.

Type 'A' cells

These are largest among all the cells found in the body of the parasite, each ranging from 30-50 x 40-60 µm in size (Pl. X, 1,2; XI, 1-9), with a large centrally placed nucleus, about 15 to 20 µm in diameter, possessing a single large central nucleolus. The cytoplasm distinctly appears vacuolated which imparts a characteristic shape to these cells and this has been reported by Shyamasundary & Rao (1975) also. These cells generally occur in the brain and it's vicinity in smaller number than type 'B' cells. These are also found in lateral nerve cords at intervals, and specially in transverse connections of ventral and dorsal

nerves (Pl. XXV, 3,4; XXVI, 2,4). In the connections these cells do not occur concurrently with type 'B' cells.

Type 'B' cells

The second type of the neurosecretory cells are smaller in size than type 'A' and vary in size measuring from 15-20 x 20-25 µm, and with nucleus measuring about 10-15 um in diameter. These are ubiquitous in distribution in the body and exhibit homogeneous nature of cytoplasm, generally lacking vacuoles. (Pl. X, 3-9; XXV, 1; XXVI, 3,6). Although the type 'B' cells are found in every innervating region, they are relatively more abundant in the anterior ganglia and along the lateral nerves (Pl. XX, 3,5; XXVII, 1,4).

Other than the aforesaid regions, type 'B' cells are also found in the suckers, pharynx, Mehli's gland complex, cirrus sac, uterine vicinity; and also near the excretory sphincter.

Cytochemically both types show presence of NSS granules (Pl. XXV, 2,5,6; XXVI, 2,4,6), and a PAS positive substance (Pl. XXV, 1; XXVI, 5). These are probably glycoproteins. An ample quantity of RNA is found in the cytoplasm of these cells. Moderate amount of protein (Pl. XXV, 3) which is chiefly basic,(protein)also appears to be present (Pl. XXVI, 3). A lipoid moiety also occurs in a good amount. The

cytochemical nature is almost similar to that already described by Shyamasundari and Rao (1975) except the nature of PAS positive substance which in the present study has been detected as diastase resistant, while Shyamasundari and Rao mentioned this moiety as liable to the diastase digestion. However, these studies support the hypothesis led by Bern and Hagadorn (1965), according to which the phenomenon of neurosecretion is nearly ubiquitous among metazoan animals. The author also agrees that there are two distinct types of neurosecretary cells as were differentiated in F. hepatica as well as in F. gigantica (Gresson & Threadgold, 1964; and Shyamasundari & Rao, 1975). The criterion of their classification adopted by Shyamasundari & Rao (1975) seems to be adequate, which have also been supported by other investigators (Kalyankar and Kankal, 1980; Kishore & Shyamasundari, 1980; and Venkata Ramakrishna et al. 1980).

The details of various histochemical tests performed on the nervous system and the neurosecretory cells of F. gigantica have been furnished in Table VI.

TABLE - VI

Results of various histochemical tests performed on the nervous system of *Fasciola gigantica*

Tests performed	Ganglia	Nerves	Neurosecretory cells "A"	Neurosecretory cells "B"
PAS	++	++	+	+
PAS, after diastase digestion	++	++	+	+
Best's Carmine	-	-	-	-
Best's Carmine, after diastase digestion	-	-	-	-
Alcian blue	-	-	-	-
Mercurybromophenol blue	++	++	++	++
Acid Solochrome cyanine	++	++	+	+
Pyronin Y, Methyl green	-	-	++ Pink	++ Pink
Sudan black B	++	++	++	++
Acetone sudan black B	-	-	-	-
Indoxyl acetate	+++	++	+	+
Indoxyl acetate, after Eserine treatment	-	-	-	-
Acetylthiocholine iodide	+++	++	-	-
Acetylthiocholine iodide, after Eserine treatment	-	-	-	-
Bargman's, Crhrome haematoxylin & phloxine	++	-	+++	+++

+++ = intensely stained; ++ = moderately stained;
+ = slightly stained; - = no stain.

VI. THE EXCRETORY SYSTEM

The excretory system in the digenetic trematodes generally consists of a median excretory bladder which is joined by a variable number of collecting ducts which further remify into smaller ductules and fine capillary vessels, each terminating at a flame cell. The excretory vesicle communicates with the exterior through a median excretory pore situated at the posterior tip of the worm.

The excretory system of trematodes was first delineated by Fraipont (1880) who observed flame cells in adult forms. In an extensive paper, Kawana (1950) described the development of the entire excretory system in cercarial stages of F. hepatica. Some histochemical studies were made on the adult excretory system of F. hepatica by Stephenson (1947d). The fine structure of flame cells was first investigated by Kummel (1959 & 1960) in the miracidium of F. hepatica and, Pentelouris and Threadgold (1963) described the occurrence and ultrastructure of flame cells in the tissue slices of adult F. hepatica. Such studies however, have not so far been made on Fasciola gigantica.

The excretory system of F. gigantica essentially does not much deviate from that of F. hepatica as it comprises a median excretory vesicle, extending approximately two third

antero-posteriorly of the entire body length. The width of the vesicle markedly varies, depending on whether it is filled on empty. The anterior end of the vesicle is bifid (Pl. XII, 1) and each of the branch recieves main collecting tubes which further branch into finer collecting tubules and finally ramify into the excretory tubules. The network resulting from the ramification of the anterior main collecting tubes is related to the one third (anterior) region while many other main collecting tubes following similar ramifying pattern appears to join directly the excretory vesicle. On either side of the vesicle, approximately 12 main collecting tubes open at intervals.

The excretory pore is provided with a well developed Sphincter (Pl. XIX, 2; XXIX, 4), which is innervated by lateral efferent branches of the ventral longitudinal posterior nerves (Pl. IX, 1). This pore appears roughly round in cross section, whose lumen is lined with a tegumental layer which is about 20 μm in thickness and continues into the excretory vesicle upto an approximate height of 0.5 mm, starting from the excretory pore (Pl. VII, 3; XII, 1; XXIX, 3). This tegumental layer is in continuity with the general body tegument. The surface of this tegument is thrown into longitudinal valleys which are probably analogous with the transverse valleys of general tegument as described in Chapter I. This tegument is followed by a thick amorphous

basement layer which in further followed by circular and longitudinal muscle layers like that of the general body tegument. The subtegumental cells are also arranged in clusters along the entire region where the vesicle lumen is lined by the tegument (Pl. VII, 3). The sphincter region exhibits an intense acetylcholinesterase activity (Pl. XXIX, 3,4). In case where the excretory vesicle was found filled with fluid the pore with sphincter was observed to be in an evaginated condition with open excretory pore, while in the empty and collapsed vesicle the sphincter was found invaginated, with the pore closed, depicting the notched posterior tip. In addition to the circular, the longitudinal muscles, dorsoventral muscles from all sides are also attached to the sphincter region (Pl. VII, 3).

The lumen of the vesicle, collecting tubes and excretory tubules are cellular with attenuated epithelial cells. The nuclei are oval, small, or elliptical, each measuring about 2 µm in diameter. The epithelium rests on a comparatively thin basement membrane which is followed by thin muscle layers. In addition to these muscle layers, dorsoventral muscles are also attached to the vesicle at places on the corresponding dorsal and ventral sides (Pl. XII, 2).

The end of fine collecting tubules are usually bifid. The termini of the excretory tubules are equipped with the flame cells (Pl. XII, 3-5). Each flame cell approximately

measures about 10 x 20 µm, with a round nucleus, having hyaline neucleoplasm. The base of the cell body is provided with flagellar tuft into the lumen of the basal stalk of the flame cell.

In the epithelium of excretory vesicle lipoid depositions were detected histochemically(Pl. XIX, 6). Same moiety was also observed in the tubules and the flame cells.

The details of various histochemical tests performed on the excretory system have been furnished in Table - VII.

The excretory system of F. gigantica however, follows the same general plan as was described in F. hepatica (Kawana, 1940), with a slight exception of the approximate number of the main collecting tubes opening into the vesicle. Perhaps the number may be variable according to the length of the parasite viz., the length of the excretory bladder.

The bifurcation of the excretory vesicle at it's anterior end presumably reveals the remnant feature of the confluence of the two distinct tubes in cercarial stage and might be having same course of development as in F. hepatica (Kawana, 1940).

The cellular lining of the excretory system also confirms the nature of the system as a close system lined with definite epithelia, rather than a simply loose fluid filled net-work as has often been described in some other digenetic trematodes (Willey, 1930; Dawes, 1946).

The function of the excretory pore appears to be regulated by a need-based neuromuscular activity, as already evidenced by the intense acetylcholinesterase localization in this region.

The excretion of fat globules has already been demonstrated in F. hepatica (von Brand & Weinland, 1924) and has been histochemically confirmed by Stephenson (1947d). The present studies also confirm the views of Stephenson (1947d) that fat is excreted through the excretory system of trematodes. Prenant (1922) also presumed the excretion of fat and mentioned the large fat droplets in the cells are released into the lumen by the cell wall rupturing locally.

TABLE - VII

Results of various histochemical tests performed on the excretory system of *Fasciola gigantica*

Tests performed	Excretory Vesicle	Collecting tubes	Excretory tubules	Flame cells	Excretory sphincter
PAS	-	-	-	-	++
PAS, after diastase digestion	-	-	-	-	-
Best's Carmine	-	-	-	-	++
Best's Carmine after diastase digestion	-	-	-	-	-
Alcian blue	-	-	-	-	-
Mercury bromophenol blue	++	++	++	++	+++
Acid Solochrome Cyanine	++	++	++	+	+++
Pyronine Y & Methyl green	-	-	-	-	+ Pink
Sudan black B	+++	++	++	++	+
Acetone Sudan black B	-	-	-	-	++
Silver impregnation	-	-	-	-	+
Indoxyl acetate	-	-	-	-	+++
Indoxyl acetate, after Eserine	-	-	-	-	-
Acetylthiocholine iodide	-	-	-	-	+++

+++ = intensely stained; ++ = moderately stained;

+ = slightly stained; - = no stain.

VII. THE REPRODUCTIVE SYSTEM

Except a few digenetic families (Didymozoomidae and Schistosomatidae), the trematodes are hermaphroditic. Generalized trematode reproductive system deviates from that of turbellarians in having common ovovitelline duct as a uterus retaining eggs before passing out. A yolk gland, distinct from ovary is the character of nearly all trematodes. Many of these show presence of paired or single copulatory canal, opening externally. Generally a male copulatory organ known as the cirrus is present. The digenetic trematodes usually possess paired, round testes situated side by side or tendam. Some may have single testis while many others may possess multiple testes (e.g., Gorgodera spp; Schistosoma spp.). These testes may be lobular or highly branched or even tubular. A sperm duct arises from each testis. Their sinuous part where they meet either may enlarge to form a spermiducal vesicle, entering into either a simple muscular tube or a highly muscular cirrus sac, which constitute the male copulatory apparatus. The distal part of the copulatory organ may either be unarmed or provided with spines, scales, thorns etc. This part is generally known as a cirrus. Cirrus sac may enclose a seminal vesicle and also prostate glands, still enclosed within the prostatic vesicle in it.

As a generalization a single ovary is present, which may be round lobed or highly branched. A short oviduct unites with common yolk duct and receives a Laurer's canal. This in course widens to form ootype, which is encircled by Mehlis' glands.

In the genus Fasciola, the testes are paired, profusely branched, tandem, occupying most of the part of postovarian intervitellarian fields. A muscular cirrus sac is antero-dorsal to the acetabulum. The genital pore is situated at the intestinal bifurcation. The ovary is dendritic, submedian, and pretesticular in position. The Laurer's canal is present whereas a seminal receptacle is wanting. The vitellaria are numerous small follicles, extending in lateral fields both dorsal and ventral to the intestinal caeca below the base of cephalic cone upto extreme posterior end of the body. The uterus is coiled, rosettes in between the acetabulum and ovary.

The reproductive system in Fasciola gigantica essentially does not much deviate from its general pattern as described in F. hepatica. The description follows:

Male reproductive system

The male reproductive system of Fasciola gigantica comprises a paired, tandem, multi-branched testes situated

slightly ventral to other organ systems (Intestinal caeca). The two testes occupy nearly 2/3 of the entire body area in the middle region, appearing as distinct oval region (Pl. I,1). Each of the testis branches into 4-5 main branches on either side which further ramify into finer branches. A fine sperm duct arises from the middle portion of each testis leading anteriorly, passing dorsally to the acetabulum, opening slightly postero-dorsally into the seminal vesicle separately and extending into the cirrus sac (Pl. XIII, 1).

The cirrus sac is a muscular bag-like structure, enclosing the cirrus. It measures 0.5 x 1 mm. and is situated antero-dorsally to the acetabulum, but not extending posteriorly to the acetabulum. Among the main male copulatory organs the first one is the bilobed seminal vesicle whose both lobes directly communicate with each other (Pl. XIII,1). The posterior lobe is almost spherical while the distal (anterior) one is elongated, extending through the ejaculatory duct which passes through the cirrus. The seminal vesicle is lined by a single-celled thick epithelial layer (Pl. XXII, 4). The epithelial cells are of varied shape and size. The epithelium rests on a basal membrane, which is followed by the layer of circular and longitudinal muscles. The space in between the seminal vesicle and cirrus sac is occupied by parenchyma cells (Pl. XXII, 3), which seems to be projected with their processes towards the seminal vericle; similar

condition has been reported by Threadgold (1975) in Fasciola hepatica. He also observed typical ultrastructural junctional complexes between the epithelial cells of seminal vesicle and adjacent parenchyma cells.

The epithelial cells vary from squamous to cuboidal in shape. Their nuclei are oval and basally situated. There is present cytoplasmic RNA in these cells, thereby indicating probability of granular endoplasmic reticulum present in these cells which were also evidently reported in F. hepatica by Threadgold (1975) in electron micrographs.

Ejaculatory duct: The distal lobe of seminal vesicle sinuously continues through cirrus and is lined by a cuboidal epithelial layer (Pl. XXI, 5). The basal part of the epithelium rests on a basement layer, followed by the circular and longitudinal muscles. These cells bear round to oval nuclei which are basal in position. The cytoplasm shows RNA present moderately like the epithelium of seminal vesicle (Pl. XXI, 5).

Numerous iunicellular glands open through their projections to the ejaculatory duct (Pl. XIII, 1). These are the prostate glands (Pl. XIII, 2) each measuring about 15 µm and having a round central nucleus, 5-7 µm in diameter. They presumably pour their secretion into the lumen of ejaculatory duct through their processes. The prostate gland cells contain

certain PAS positive moiety which is non-liable to diastase digestion (Pl. XXI, 3,6). These are highly protenaceous, showing basic protein (Pl. XX, 6; XXI, 4). Fats are absent, except a small quantity of bound lipids present in it (Pl. XXI, 4).

The seminal vesicle is filled with seminal fluid containing spermatozoa (Pl. XX, 6; XXII, 4).

The cirrus and cirrus sac are covered by a syncytial tegument. The tegument of cirrus sac is comparatively thin than that over the cirrus and is devoid of spines; while the tegument of cirrus show a quincunxial pattern of spines (Pl. XIX, 5), which are smaller than the general body spines, each measuring about 15 x 20 µm.

The cirrus is protrusible and retractable from and within the cirrus sac through the metraterm, and the sides of the sac are provided with special diagonal muscles (Pl. XIII, 1). The base of the cirrus is provided with a strongly muscular sphincter which, on the either side exhibit a distinct nerve plexus (Pl. XIII, 1). The region of the nerve plexus and sphincter have shown the presence of Acetylcholinesterase activity (Pl. XXIX, 5).

Female reproductive system

The female reproductive system comprises single, branched and dendritic ovary, occupying about 23 x 4 mm^2 area

on the right side of the worm to the midline, posterior to the ventral sucker (Pl. I, 2). The ovary generally comprises 3 main digitiform dendrites and which further branch (bifurcate). Post-median main trunk gives rise to a short oviduct traversing into the Mehlis' gland complex and opens to join the ovovitelline duct in between the vitelline reservoir and the ootype (pl. XIII, 5). The ootype is the main chamber where the mature oocytes and vitelline components come in contact. The ootype measures about 0.1 x 0.4 mm and is an elliptical structure, lined with an epithelial layer (Pl. XIII, 5). This epithelium, like in other organs, rests on a thin basement membrane and is followed by thin muscle layer. The junction of the ovovitelline duct and ootype is provided with a very short diverticulum and on the inner side an oviducal sphincter (Pl. XIII, 5). The distal end of the ootype leads into the uterus and the junction is guarded by a distinct uterine valve (Pl. XIII, 5). This structure has already been reported by Rao and Madhavi (1962) in the same worm. The uterus traverses anteriorly and opens through the female pore on the left side of metraterm cavity. The gonopore is also guarded by a muscular sphincter (Pl. XIII, 1; XX, 6). The entire uterus is also divisible into two parts on the basis of it's histological details (i) the proximal uterus runs from the ootype upto some distance, it's lumen is lined with an epithelium whose cells are of irregular shape and vary from cuboidal to columnar (Pl. XIII, 6). While the distal part rossettes

in between ovary and acetabulum, it is lined with an attenuated epithelium. These epithelia are resting on a basement membrane, followed by a layer of muscles. The ootype is provided with the processes appearing radial to it from unicellular glands (Mehlis' glands), which are also of two types morphologically distinct cells, type-I (MGC 1) and cell type-II (MGC 2). Type-I cells are larger, appearing polygonal in shape, closely apposed, each measuring about 20 µm and having distinct nucleus (Pl. XIII, 3). The cell, type-II are smaller, pear-shaped, measuring about 10 µm and occur in the inner region of the gland complex (Pl. XIII, 4,5). The cell type-I possesses certain non-glycogen PAS positive substance (Pl. XXII, 5; XXVII, 3), while this moiety is lacking in type-II cells. The other histochemical characters are presence of cytoplasmic RNA in type-I cell and absent in type-II cells (Pl. XXIII, 4). This RNA is confined to the perinuclear region only. Some bound lipid is present in type-I cell, and absent in type-II cells (Pl. XXIII, 6), and there is no lipid moiety in both of them. The protein level is either very low or nearly negligible in both cells (Pl. XXIII, 1,5). The entire complex acquires a thick spherical structure measuring about 1 mm in diameter.

The short sinuous, delicate Laurer's canal arises from the ovovitelline duct (Pl. XIII, 5), and after traversing a short distance antero-dorsally, opens on the dorsal side of the body. The opening has been observed clearly in frontal

sections (Pl. V, 5), and is provided with a strech of tegument. The tegument in this region proceeds into the canal upto some distance, having all the general characters of the tegument except spines. The tegument is followed by the basement layer, circular and longitudinal muscles and underneath, the sub-tegumental cells, arranged in clusters, appearing radially around the opening (Pl. V, 5).

Vitelline glands

The vitellaria (vitelline glands) occupy entire lateral field on either side of the testes as well as the caudal region of posterior testis (Pl. I, 1).

The vitelline cells are developed within the vitelline follicles. Immature cells are developed from stem cells of the follicle wall. These cells vary in shape from oval to round each measuring 15-20 µm, with a large central nucleus, 5.0 - 7.0 µm in diameter, with generally a single nucleolus (Pl. XV, 4-8). The nucleoplasm contains dense chromatin granules.

The cytoplasm is highly granular, showing ample cyto-plasmic RNA (Pl. XXIII, 2), probably indicating the process of continuous protein synthesis. When cells mature, they tend to move in the central region of the follicle and become much enlarged, each measuring 30-35 µm in diameter, with a round nucleus, about 7 µm in diameter with a smaller nucleolus as

compared to those in the immature cells (Pl. XV, 4-8).

The RNA is found in lesser amount in these cells and a dense proteinic moiety appears (Pl. XXVIII, 5), which is probably the precursor of the vitelline globules. These globules are arranged peripherally in the fully matured cell. These cells also contain fairly good amount of glycogen (Pl. XXVIII, 3). The protein in the mature cells, particularly in the vitelline globules is strongly basic as it gives an intense reaction with Acid Solochrome Cyanine (Pl. XXVIII, 2).

The mature cells move and are liberated into the lumen of collecting vitelline ducts and aggregate in the vitelline reservoir. Invariably the main vitelline ducts are found packed with vitelline cells (Pl. XXVIII, 2).

In the vitelline follicles few cells are small in size and irregular in shape, each measuring about 10 µm. These are arranged around the developing vitelline cells. The nucleus is also of oval to round shape with indistinct nucleolus. These are probably the nurse cells as were described by Irwin and Threadgold (1970) in F. hepatica.

At the base of the Mehlis' gland complex lies the pyriform vitelline reservoir, which receives the transverse vitelline ducts from either side. Each of the transverse vitelline ducts starts from the antero-lateral margins of the anterior testis, which seems to bifurcate anteriorly and

posteriorly. The anterior branches reach upto the base of cephalic cone, while the posterior one traverses nearly parallel to the outline of the lateral margin of the posterior testis. Both the posterior longitudinal vitelline ducts meet near the posterior extremity in the form of a caudal bridge (Pl. V, 2); XXVIII, 1). At places these vitelline ducts also form loops in the posterior field (Pl. V, 2). The lateral vitelline ducts are supplied with numerous fine ramifield tubules which meet the vitelline follicles (Pl. XXVIII, 1). The vitelline follicles are situated on ventral as well as dorsal sides, disposed in V-shape on lateral margins in the T.S. of the worm (Pl. VII, 4).

The vitelline duct is lined by a flattened epithelium, the like of which has also been observed in F. hepatica by Irwin and Threadgold (1970).

The fact that the vitelline cells contribute to the trematode egg-shell was first realized by Leuckart (1886) while some of his contemporaries believed that the Mehlis' gland was solely responsible for this purpose (Sommer, 1880; Looss, 1885; Schubmann, 1905). However, Leuckart's view has since been confirmed by many workers, chiefly Dawes (1940), Stephenson (1947c); Smyth & Clegg (1959), Burton (1963) and Irwin & Threadgold (1970). The concept of vitelline cells production in the vitelline follicles was established much earlier, but little work was done in this regard particularly

in regard to their cytomorphosis. Stephenson (1947), Smyth (1951), Smyth and Clegg (1959), and more recently Irwin and Threadgold (1970) on the morphology of vitelline cells and concluded that the vitelline cells are found in various stages of development in vitelline follicles. The present author has also arrived at the same conclusion.

Gonadal anomalies

During present studies two flukes showed gonadal anomalies; one was a monorchid specimen and the other one had a duplicate ovarian complement, one ovary on either side of the anterior body region. The details of these anomalies are as follows:

Specimen 'A':- The fluke measured 3.25 cms in length and had single anterior testis occupying a space of about 7.5 mm in length and 4.35 mm in maximum width. The posterior testis perhaps atrophied or had not developed at all since the space, about 9.75 x 3.0 mm in expanse was occupied by depleted parenchyma only. The fluke was obviously a monorchid one (Pl. V, 3).

Specimen 'B':- In this specimen which measured about 4.0 cm in length, the dendritic ovary appeared duplicated and bilaterally disposed, one of the ovaries was dextral and the

other sinistral in position. The left ovary measured 2.8 x 1.94 mm and the right one measured 3.15 x 2.25 mm. Each of these ovaries comprised three main branches L1, L2, L3 and R1 R2 and R3 respectively; the three branches having arisen from a basal rachis which continued posteriorly as the oviduct on either side. The ovarian branches appeared bifid terminally. The ovaries, however, appeared mature and the uterus was packed with fully developed eggs. There was a single ootype and a single vitelline reservoir (Pl. V, 6).

Although structural anomalies in the Digenea have been frequently reported over (Alekseeve, 1962; Macy, 1950; Madhavi & Rao, 1972; Manter, 1927; and Price, 1930) those in fasciolids have been rather scarce. Gallego-Berringer (1952) has reported the unilateral occurrence of vitellaria in an anomalous specimen of Fasciola hepatica and Healey (1955) has described the sinistral position of ovary in another specimen of the same species but as far as the writer is aware, structural anomalies, particularly those relating to the reproductive system have not been recorded in Fasciola gigantica and this is perhaps the first report in this regard.

Gross features of spermatogenesis

The spermatogenesis in digenetic trematodes attracted attention of earlier workers, though only gross features could be described. Major contributions in this field are those

by Cable (1931), Yosufzai (1952a), Dhingra (1954), Dunn (1959), Burton (1960) and Gresson (1957). Spermatogenesis in Fasciola gigantica essentially followw the general metazoan pattern. In the present study the obvious stages of spermatogenesis have been observed in sections.

The spermatogonia occupy a peripheral position in the testis and are usually in contact with its external wall (Pl. XIV, 1,2). The spermatogonia constitute about three cell generations, primary, secondary and tertiary, The four tertiary spermatogonia divide mitotically resulting in a group of eight primary spermatocytes (Pl. XIV, 3,4). These are united centrally with a cytoplasmic rachis. This eight cell stage grows in size from 15 um upto 30 um in diameter as each cell grows from 5 to 10 um in diameter (Pl. XIV, 4-6). These stages are most stable, and quite many. Here the first meiotic division occurs, resulting in a group of sixteen secondary spermatocytes which are haploid in number (Pl. XIV, 6, 7). The secondary spermatocytes appear in deeper part of the testis. These sixteen cell stage followed by the second meiotic division gives rise to a cluster of thirty two spermatids (Pl. XIV, 8). These are transformed into spermatozoa. This stage is short, stable and the nuclei of each spermatid immediately undergoes elongation and ultimately moves towards the base of the spermatid and becomes rod like, often coiled, and filiform, enclosing some cytoplasm (Pl. XIV, 9, 10).

Each spermatid which appears to be attached with the central mass of cytoplasm measures about 4 x 10 µm. Primary spermatogonia and spermatocytes are RNA-rich, spermatid is DNA rich, particularly the rachis of cytoplasm. This DNA rich material may probably be regarded as the nuclear material, which was reported by Yosufzai (1952) in F. hepatica, However, all other features are similar to those reviewed by Gresson (1965). The ripe ♂permatozoan contained within the seminal vesicle appear to posses ample glycogen which is possibly utilized for the mobility of the sperms.

Oogenesis:

The oogonia are situated near the periphery of the ovary. Each of the oogonia and its nucleus measure 10 and 5 µm in diameter respectively (Pl. XV, 2). The nucleoplasm of the oogonia is some what featureless and the nucleoli are either one to two, indistinct or absent. This fact perhaps confirms the hypothesis led by Yosufzai (1953♂). According to him the nuclear materials including chromatin and nucleoli are extruded from the nucleus into the cytoplasm. These cells are also RNA rich, which also confirms the presence of granular endoplasmic reticulum in these cells as were described in F. hepatica (Gresson, 1962). The mature oocytes measure about 25 µm with round nucleus measuring 10 µm in diameter with

generally one nucleolus (Pl. XV, 2). The cytoplasm shows fat moiety (Pl. XXIV, 6) and is RNA rich (Pl. XXIII, 4). Govaert (1953a,b; 1954; 1955 & 1960) also observed DNA in these cells in F. hepatica by Feulgen staining. He also concluded that the DNA content remains constant during the oogenesis.

Eggs

The mature eggs are oval and operculate, each measuring 35 x 50 µm (Pl. XV, 11), The eggs are glycogen rich (Pl. XXII, 1,2; XXVIII, 4). This glycogen is localized in the vitelline cells whereas the egg shell appears rich in basic proteins, as indicated by Acids Solochrome Cyanine R. (Pl. XXI, 1,2; XX, 6). The oocyte lies within the egg near the opercular side surrounded with the packed mass of vitelline cells (Pl. XV, 9,10).

The histochemical responses of various components of the reproductive system to various tests are indicated in the Table VIII.

TABLE - VIII

Results of various histochemical tests performed on the reproductive system of Fasciola gigantica.

Test performed	Prostate gland cells	Sperms	Testicular follicules	Ootype wall	Oogonia	Mature Oocytes	MGC_1	MGC_2	Vitelline Cells (Immature)	Vitelline Cells (Mature)	Vitelline Globules	Eggs	Uterine wall
PAS	++	+	+	-	-	-	+	-	-	+++	+++	+++	+
PAS, after diastase digestion	++	-	-	-	-	-	+	-	-	-	-	-	+
Best's Carmine	-	+	+	-	-	-	-	-	-	+++	+++	+++	-
Best&s Carmine, after diastase digestion	-	-	-	-	-	-	-	-	-	-	-	-	-
Alcian blue	-	-	-	-	-	-	-	-	-	-	-	-Rs	-
Mercury bromophenol blue	+++	++	++	++	++	+++	+	+/-	++	+++	+++	++	++
Solochrome Cyanine	-	-	+	-	+/-	*/-	+	-	==	+++	+++	+++	++
Pyronin Y & Methyl green	++ Pink	- Pink +++ green	++ Pnk +++ Green	+++Pink	+++ Pink	+ Pink	++Pnk	-	+ Pink	+++ Pink	-	-	++ Pink
Sudan black B	+	-	-	-	+	+	-	-	-	-	-	-	-
Acetone Sudan black B	+	+/-	+/-	++	+	++	++	-	+	+++	-	-	+

+++ = Intensely stained; +++ = Moderately stained; + = slightly stained; - = no stain; +/- = Not known.

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LEGENDS TO PLATES

ADN	=	Anterior, dorsal nerve.
AG	=	Anterior ganglion
ALN	=	Anterior, lateral nerve.
AVN	=	Anterior, ventral nerve.
BL	=	Basement layer.
C	=	Cuticle.
CM	=	Circular muscle.
CR	=	Cirrus.
CS	=	Cirrus sac.
DM	=	Diagonal muscle.
DVM	=	Dorso-ventral muscle.
E	=	Egg.
ED	=	Ejaculatory duct.
EP	=	Excretory pore.
EPC	=	Epicuticle.
EPT	=	Epithelium.
ES	=	Egg-shell.
ESP	=	Excretory sphincter.
ET	=	Excretory tubules.
EV	=	Excretory vesicle.
EVE	=	Excretory vesicle, epithelium.
FC	=	Flame cell.
FT	=	Flagellar tuft.

GC = Ganglionic commissure.

GDC = Gastrodermal cell.

GEC = Glandular epithelial cell.

GL = Glycogen.

IC = Intestinal caecum.

LC = Laurer's canal.

LGC = Longitudinal corrugations.

LM = Longitudinal muscle

M = Metraterm.

MB = Myoblast.

MG = Mehlis' gland.

MGC_1 = Mehlis' gland cell-I.

MGC_2 = Mehlis' gland cell-II.

N = Nucleus

NA = Nerve anastomoses.

NL = Nucleolus.

NP = Nerve plexus.

NSC = Neurosecretory cell.

NV = Nerve.

OC = Oocyte.

OD = Oviduct.

OP = Oesophagus.

OPC = Operculum.

OS = Oral sucker.

OT = Ootype.

OV = Ovary.

P = Parenchyma.

PC = Parenchyma cell.

PDN = Posterior dorsal nerve.

PG = Prostate gland.

PGC = Prostate gland cell.

PH = Pharynx.

PLN = Posterior lateral nerve.

PPH = Prepharynx.

PU = Proximal uterus.

PVN = Posterior ventral nerve.

RM = Radial muscle.

S = Spine.

SC = Secretory cell.

SF = Seminal fluid.

SM = Sphincter muscle.

SPH = Sphincter.

SV = Seminal vesicle.

T = Testis.

TC = Tegumental cell.

TM = Transverse muscle.

TNB = Transverse nerve branches.

UP = Uterine pore.

UT = Uterus.

UV = Uterine valve.

V. = Valley.

VC = Vitelline cell.

VD = Vitelline duct.

VDF = Vas-defernse.

VF = Vitelline follicle.

VG = Vitelline globule.

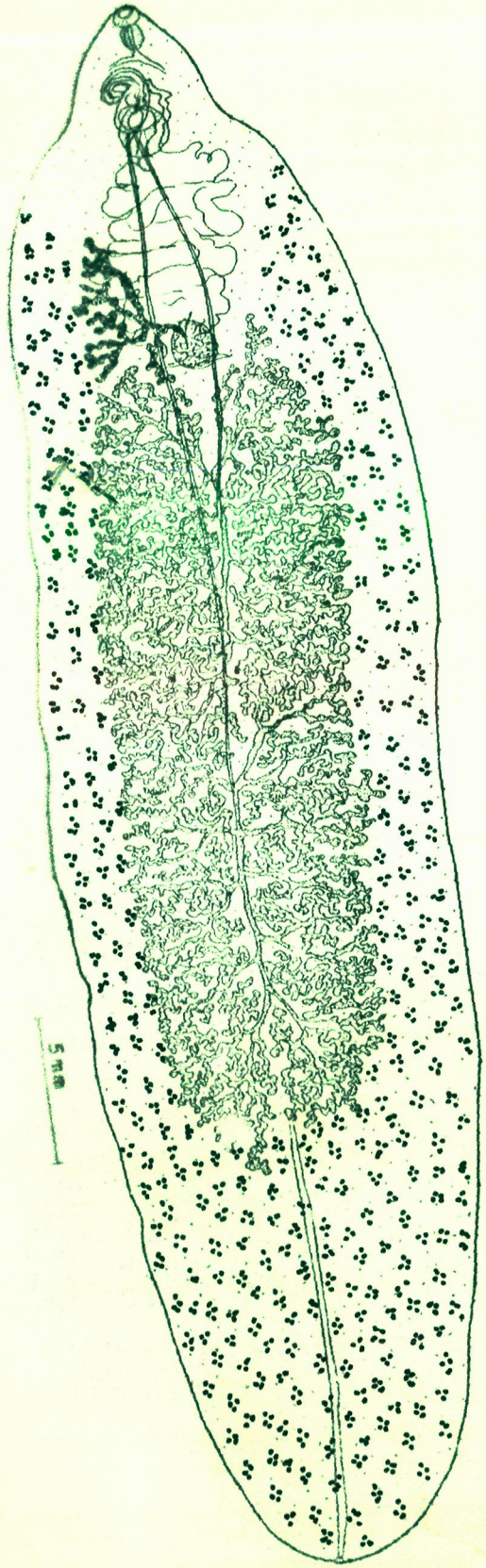
VR = Vitelline reservoir.

VS = Ventral sucker.

PLATE I

Fig. 1. *F. gigantica* : Reproductive system, *toto* mount stained with Borax Carmine. X 30

Fig. 2. *F. gigantica* : Digestive system, *toto* mount stained with Borax Carmine. X 30



1

2

PLATE I

PLATE II

Fig. 1. F. gigantica : Tegument & hypodermis, transverse section stained with Heidenhain's Azan. X 2000

Fig. 2. F. gigantica : Spines of anterior region, toto mount stained with Acetylthiocholine iodide. X 675

Fig. 3-4. F. gigantica : Tegumental secretory cells, transverse section stained with Heidenhain's Azan. X 2000

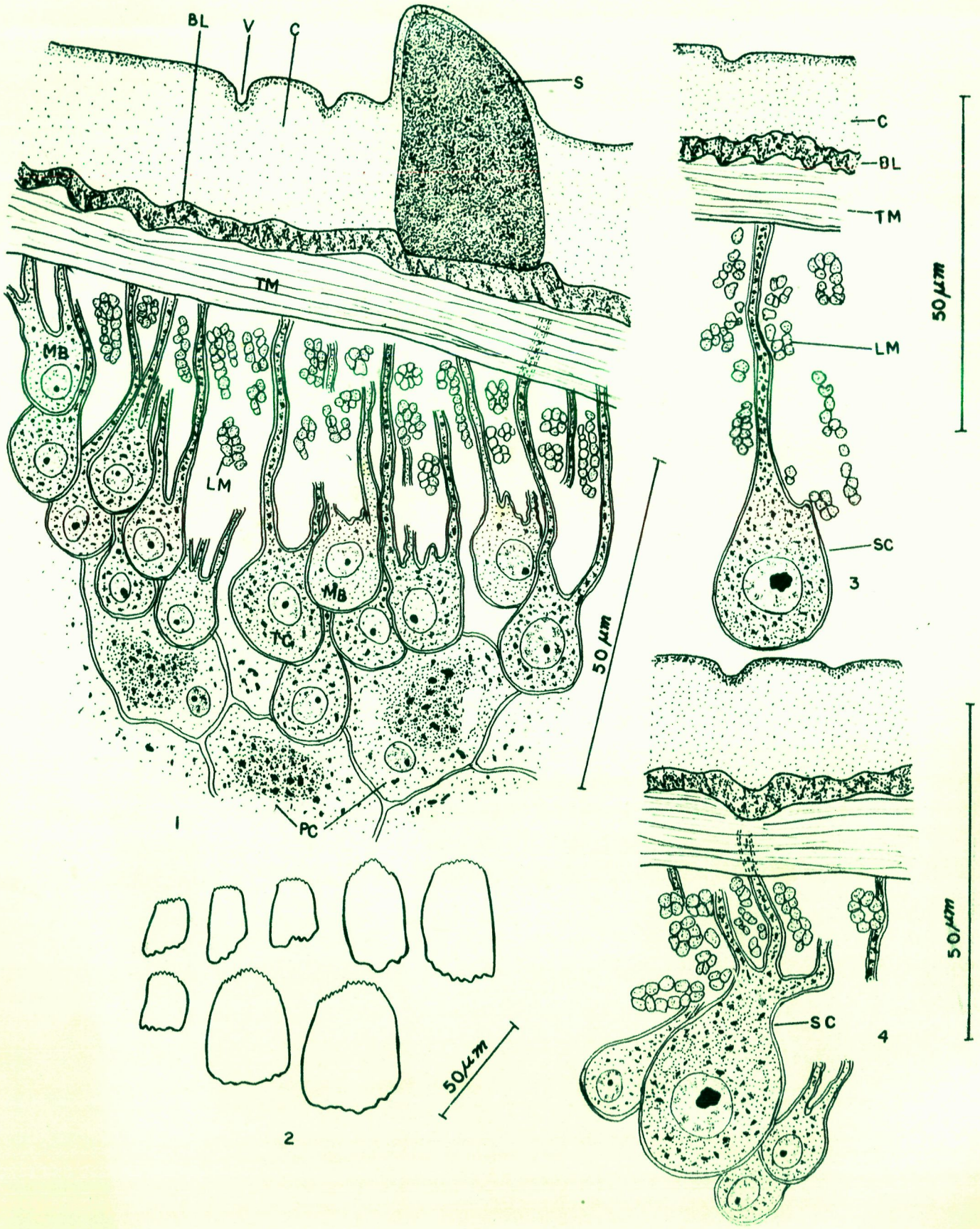


PLATE II

PLATE III

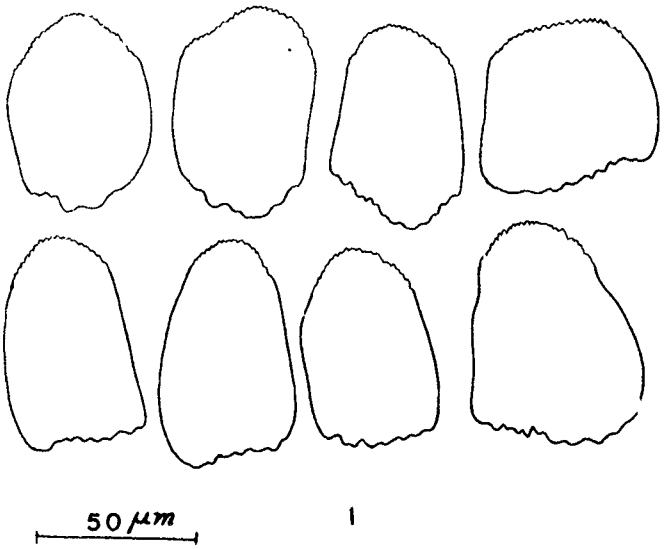
Fig. 1-3. F. gigantica : Spines of various body regions, toto mount stained with Acetylthiocholine iodide. X 675

1, spines of genital atrium region;

2, spines of post-acetabular region;

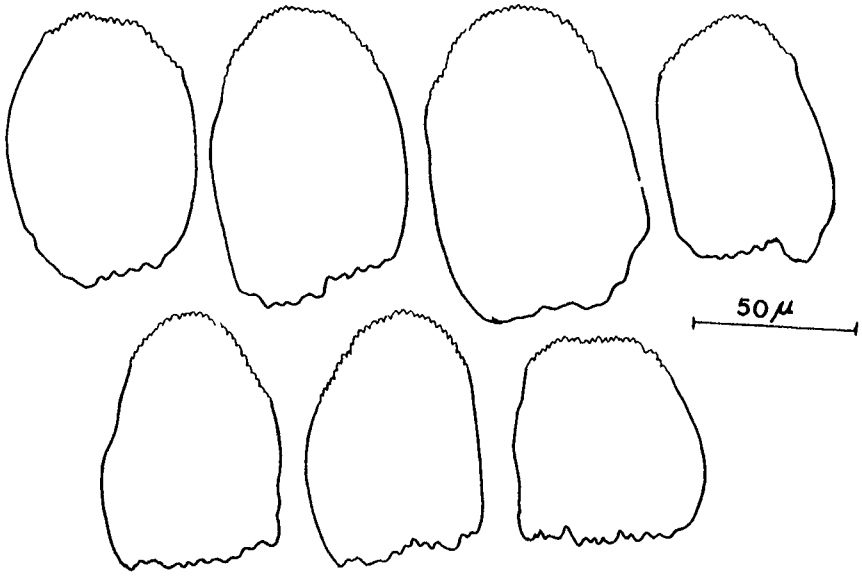
3, spines of posterior region.

Fig. 4. - F. gigantica : Direction of spines, toto mount stained with Borax Carmine. X 60



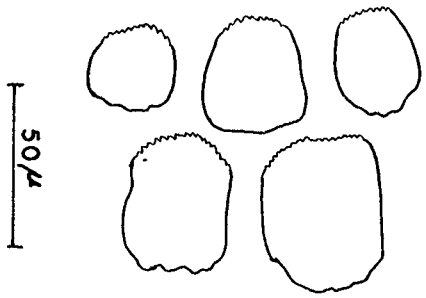
50 μm

1



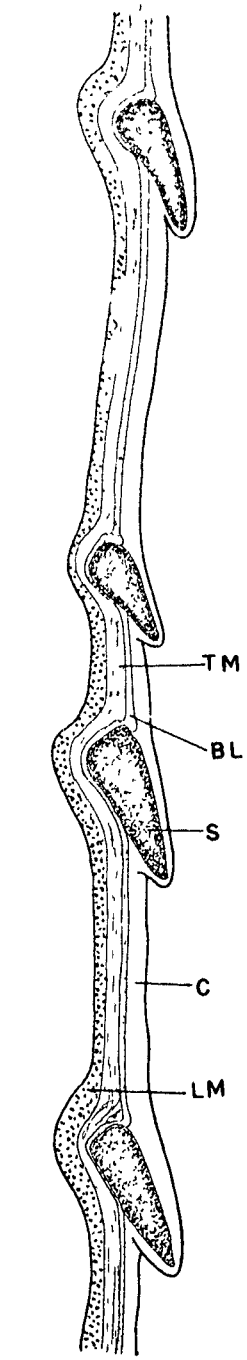
50μ

2



50μ

3



4

100μm

PLATE III

PLATE IV

Fig. 1-3. F. gigantica : Parenchyma, frontal sections stained with (PAS). X 1000

Fig. 4. F. gigantica : Nuclei of parenchyma cells, frontal sections stained with (H & E). X 2000

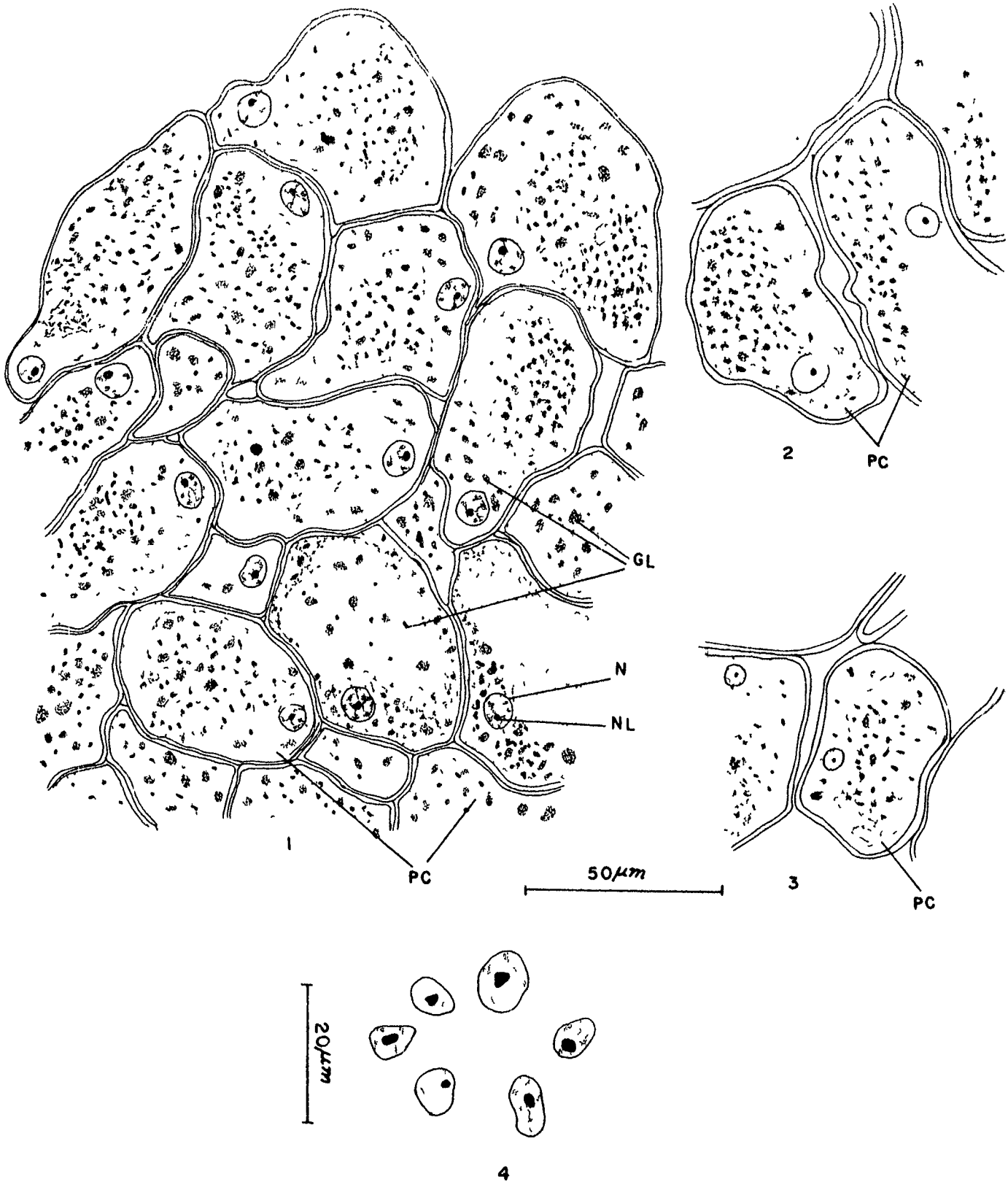


PLATE IV

PLATE V

Fig. 1. F. gigantica : Intestinal caecum, frontal section stained with (H & E). X 1000

Fig. 2. F. gigantica : Lay-out of the vitelline ducts, toto mount stained with Indoxyl acetate. X 15

Fig. 3. F. gigantica : Anomalous fluke, monorchid form, toto mount stained with Borax Carmine. X 15

Fig. 4. F. gigantica : Gastrodermis, frontal section stained with (H & E). X 2000

Fig. 5. F. gigantica : Opening of Laurer's canal, frontal section stained with (H & E). X 2000

Fig. 6. F. gigantica : Anomalous fluke with duplicate ovary, toto mount stained with Borax Carmine. X 15

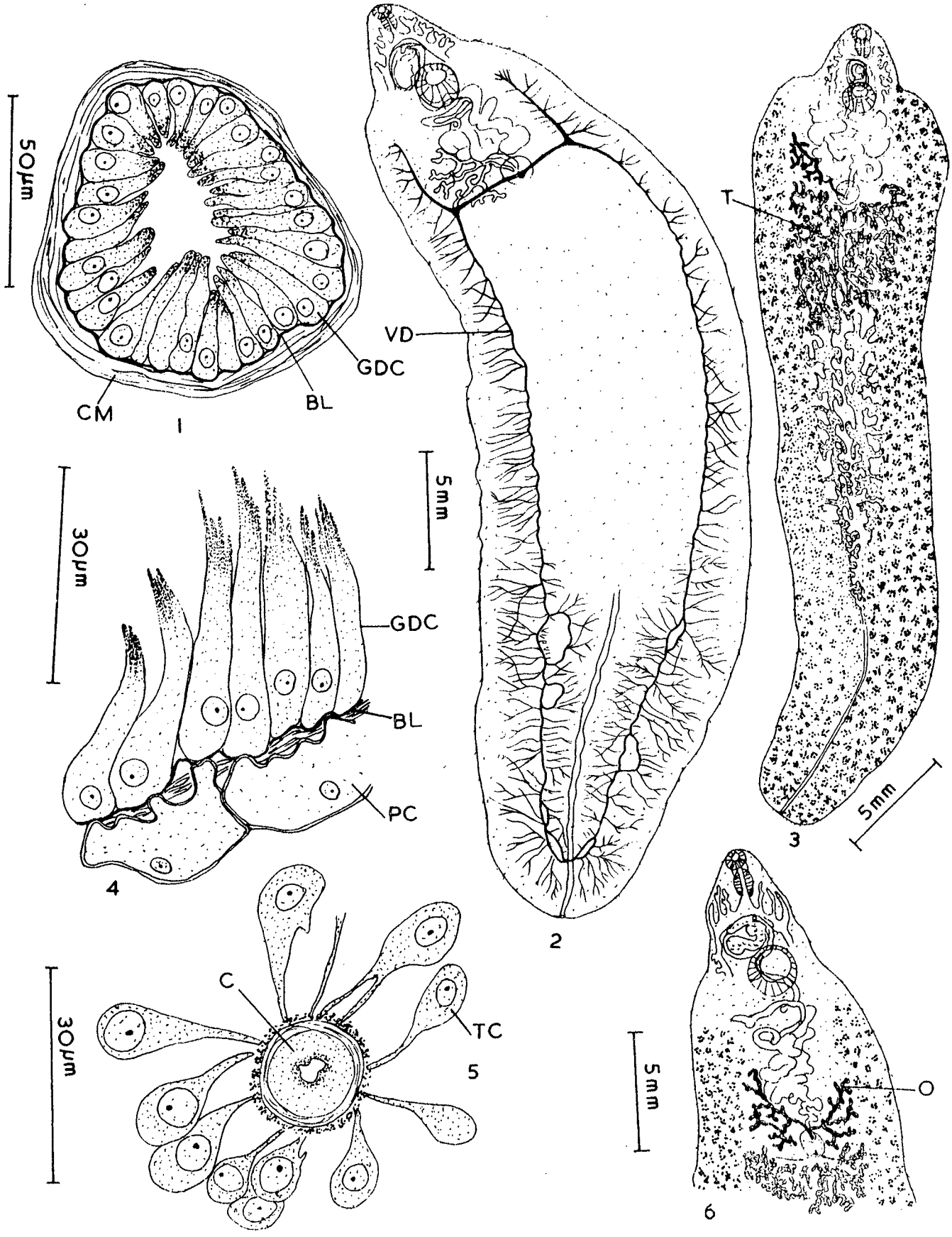


PLATE V

PLATE VI

Fig. 1. F. gigantica : Lay-out of anterior diagonal muscles through reconstruction, frontal sections stained with (H & E). X 60

Fig. 2. F. gigantica : Anterior digestive system through reconstruction, frontal sections stained with (H & E). X 100

Fig. 3. F. gigantica : Lay-out of musculature in cirrus sac region through reconstruction, transverse sections stained with Heidenhain's Azan. X 60

Fig. 4. F. gigantica : Lay-out of musculature in pre-acetabular region through reconstruction, transverse section stained with Heidenhain's Azan. X 60

Fig. 5. F. gigantica : Lay-out of musculature in acetabular region through reconstruction, transverse section stained with Heidenhain's Azan. X 60

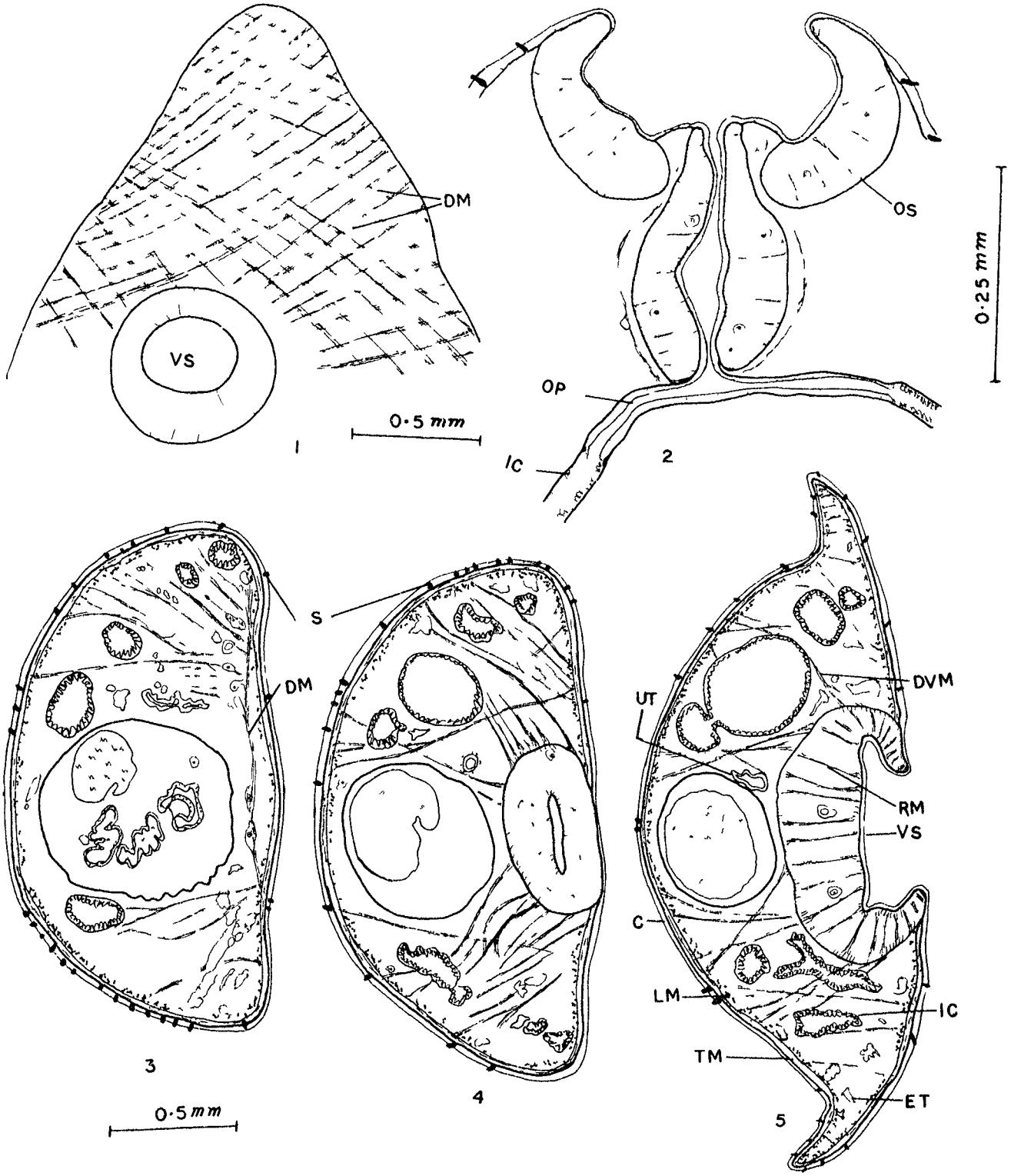


PLATE VI

PLATE VII

Fig. 1. F. gigantica : Lay-out of musculature in acetabulum through reconstruction, transverse sections stained with Heidenhain's Azan. X 100

Fig. 2. F. gigantica : Peri-intestinal muscles, frontal section stained with (H & E). X 600

Fig. 3. F. gigantica : Excretory pore, transverse section stained with Heidenhain's Azan. X 600

Fig. 4. F. gigantica : Display of various organs in uterine region, transverse section stained with (H & E). X 60

Fig. 5. F. gigantica : Single myoblast of anterior diagonal muscle, transverse section stained with Heidenhain's Azan. X 2000

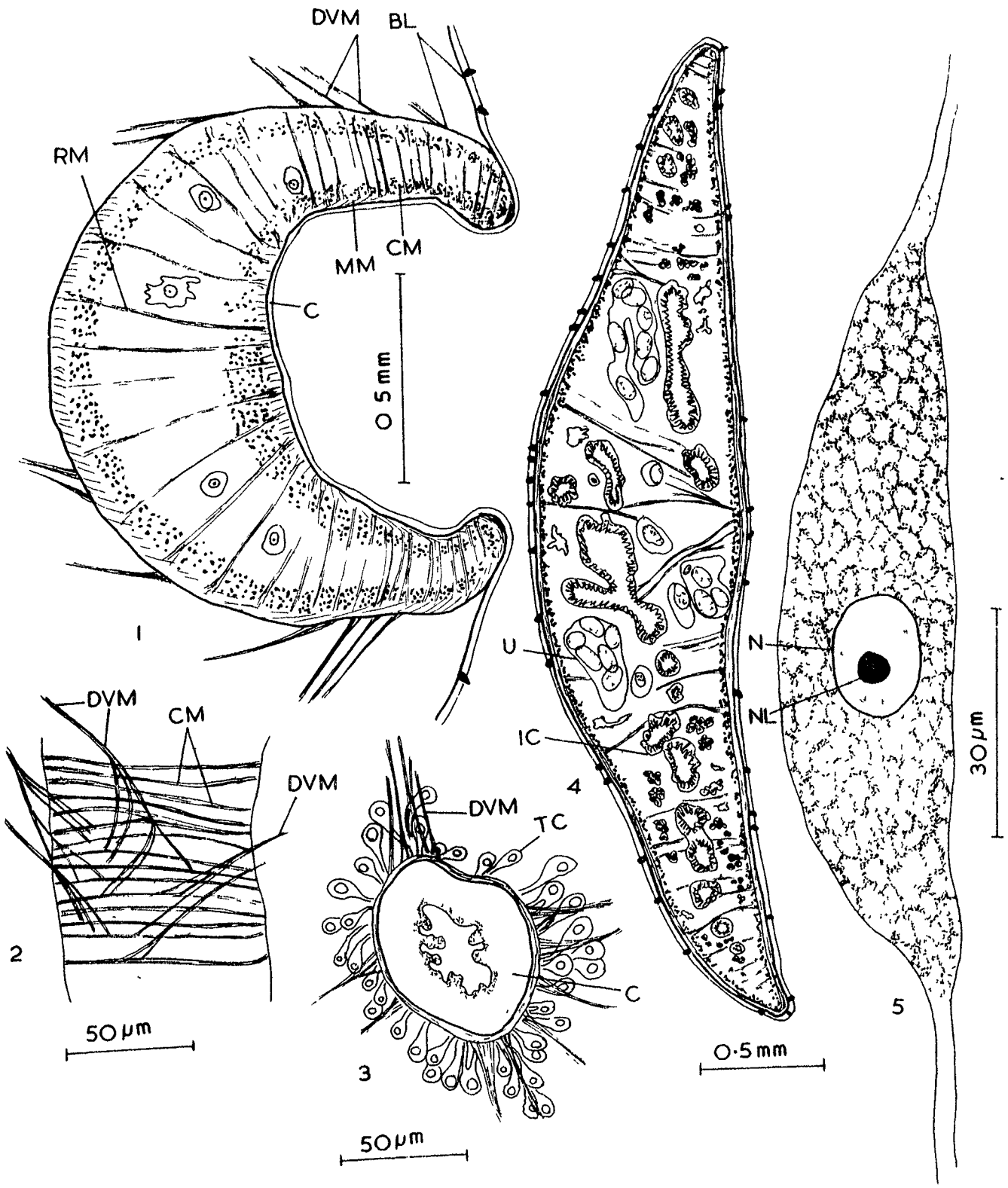

DVM
BL
RM
MM
CM
C
0·5 mm
1
U
IC
4
N
NL
30 µm
5
DVM
CM
DVM
2
50 µm
DVM
TC
C
3
50 µm
0·5 mm

PLATE VII

PLATE VIII

Fig. 1-5. F. gigantica : Fibres of longitudinal muscles attached with myoblasts, frontal sections stained with (H & E). X 2000

Fig. 6-8. F. gigantica : Fibres of radial muscles of ventral sucker, transverse sections stained with Heidenhains Azan. X 2000

Fig. 9-13. F. gigantica : Fibres and myoblasts of dorso-ventral muscles, transverse sections stained with Heidenhain's Azan. X 2000

Fig. 14-16. F. gigantica : Nerve endings in radial muscles of suckers, transverse sections stained with (H & E). X 2000

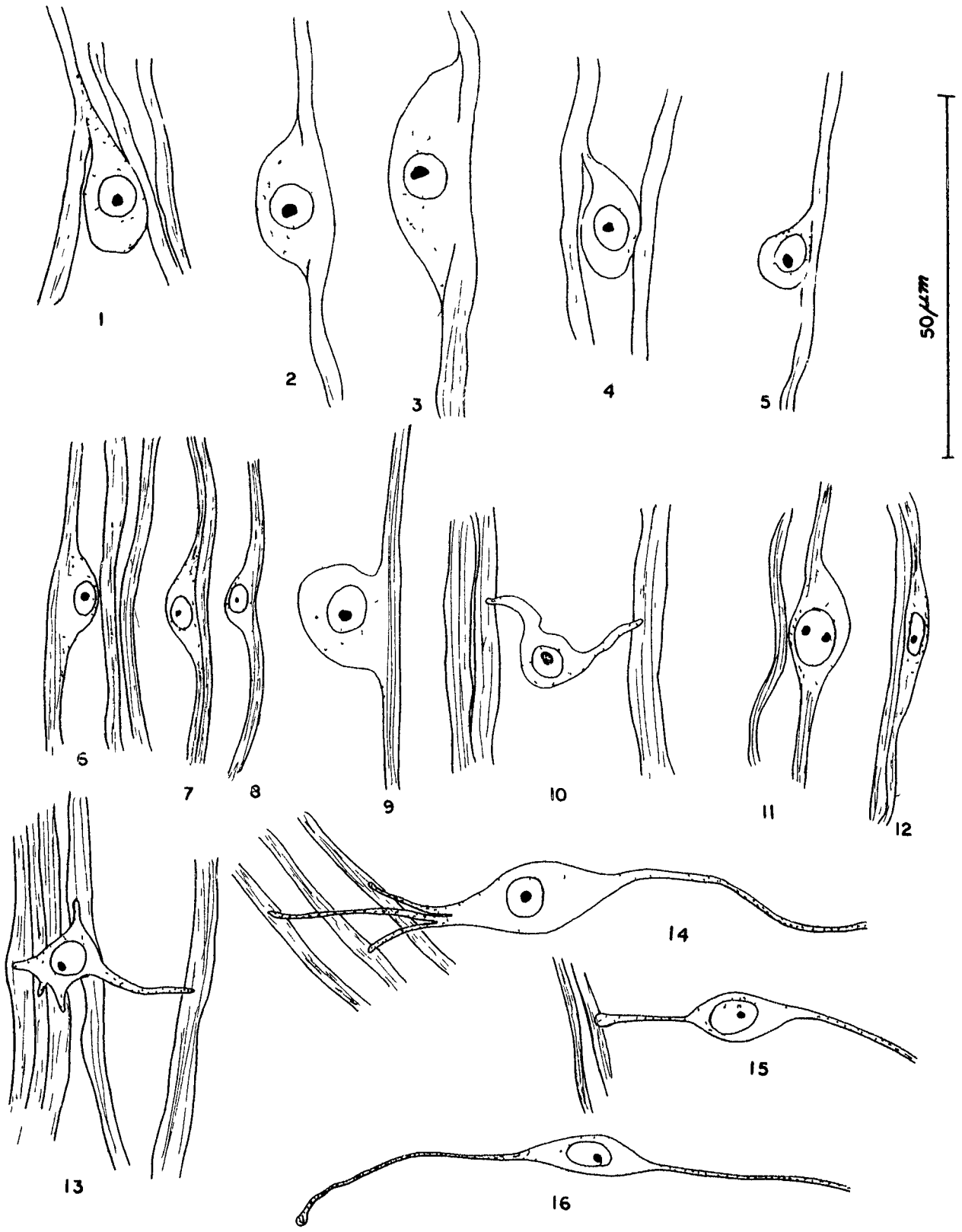


PLATE VIII

PLATE IX

Fig. 1. F. gigantica : Lay-out of nervous system, toto mount stained with Indoxyl acetate. X 15

Fig. 2. F. gigantica : Anterior nervous system through reconstruction, frontal sections stained with (H & E). X 60

Fig. 3. F. gigantica : Innervation in Mehlis' gland complex, toto mount stained with Indoxyl acetate. X 60

Fig. 4-10. F. gigantica : Atlas of anterior nervous system through reconstruction, transverse sections stained with Heidenhain's Azan. X 60

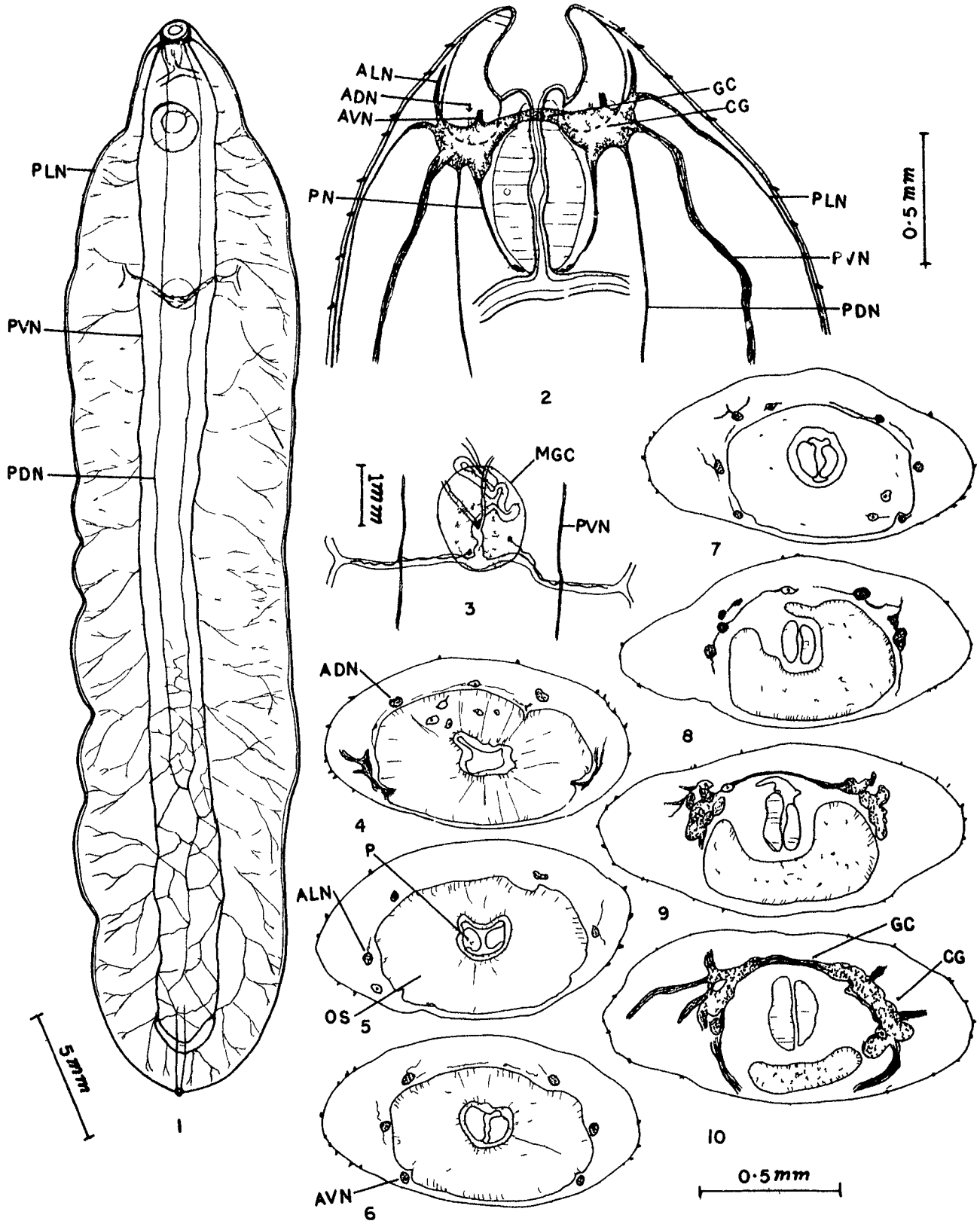

PLN
PVN
PDN
5mm
1
ALN
ADN
AVN
GC
CG
PN
PLN
PVN
PDN
0·5mm
2
1mm
MGC
PVN
3
ADN
4
P
ALN
OS
5
AVN
6
7
8
9
GC
CG
10
0·5mm

PLATE IX

PLATE X

Fig. 1-2. F. gigantica : Neurosecretory cells type "A" in the vicinity of ventral nerve, frontal sections stained with Chrome haematoxylin and Phloxin. & (PAS). X 2000

Fig. 3-9. F. gigantica : Neurosecretory cells type "B" from anterior ganglia, frontal sections stained with Heidenhain's Azan. X 2000

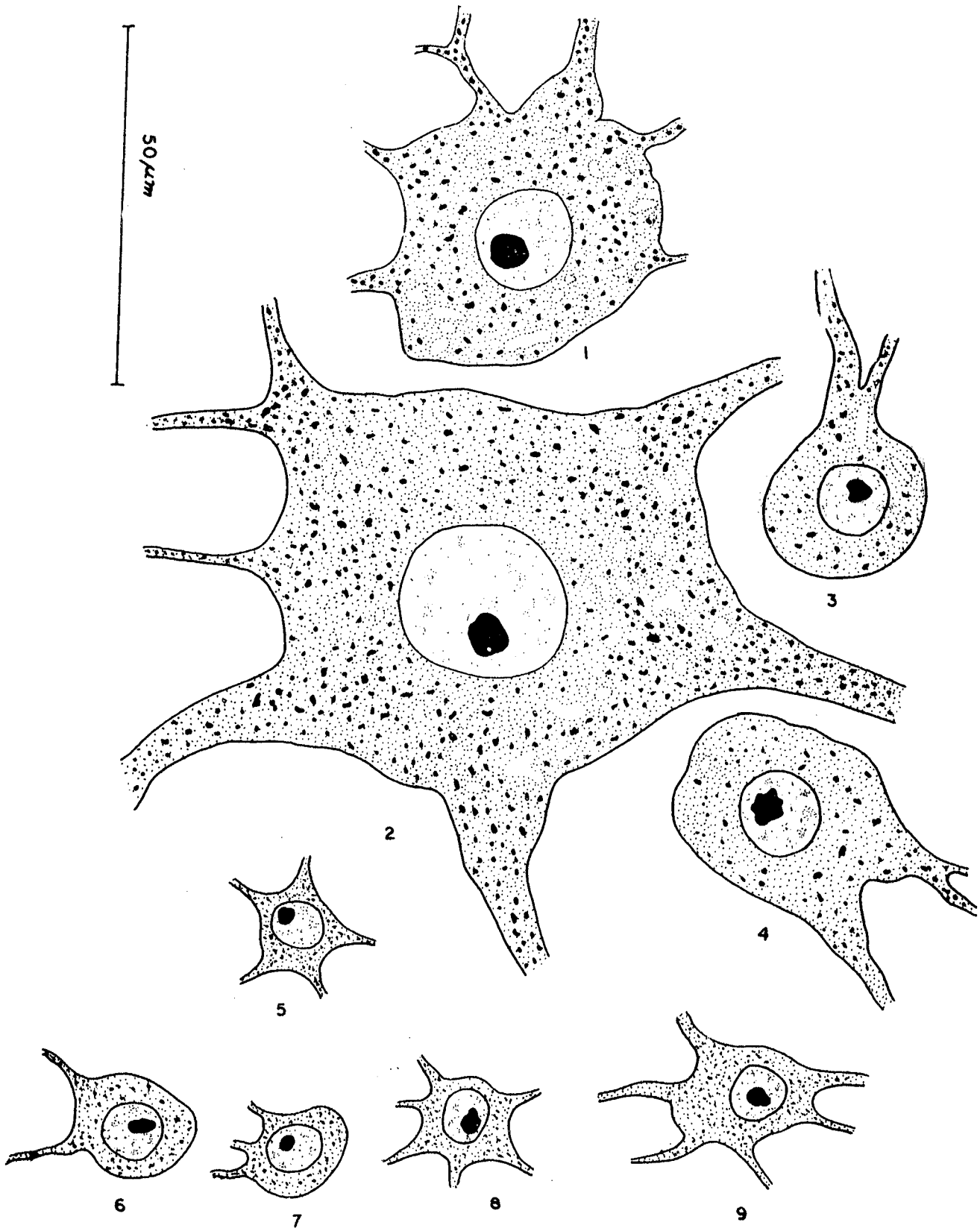


PLATE X

PLATE XI

Fig. 1-9. F. gigantica : Neurosecretory cells type "A" from various body regions, frontal sections stained with Chrome haematoxylin & Phloxin and Heidenhain's Azan. X 2000

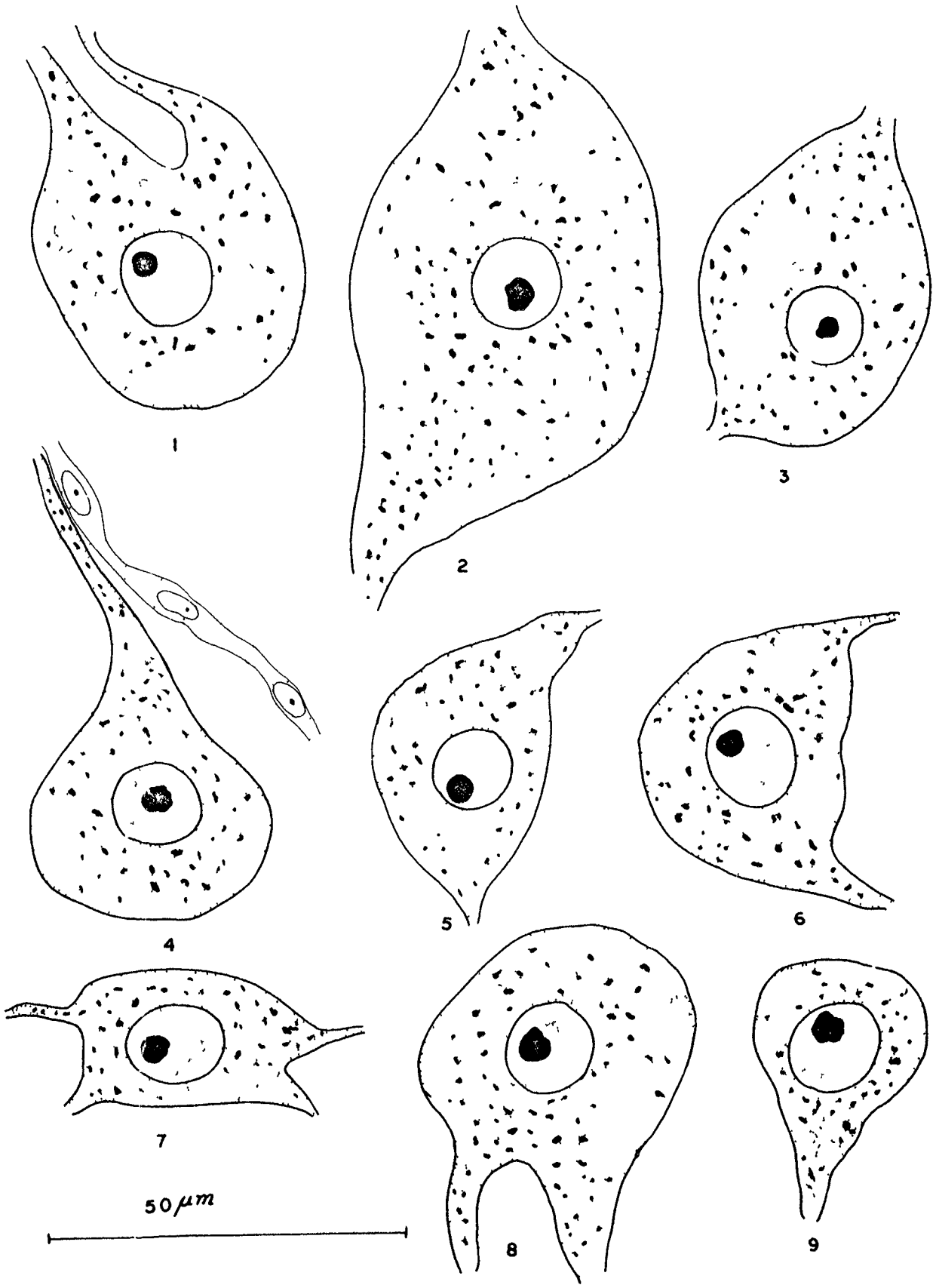


PLATE XI

PLATE XII

Fig. 1. F. gigantica : Excretory vesicle through reconstruction, frontal sections stained with Acid Solochrome Cyanine. X 30

Fig. 2. F. gigantica : Excretory vesicle through reconstruction, transverse sections stained with (H & E). X 600

Fig. 3-5. F. gigantica : Flame cells, frontal sections stained with Heidenhain's Azan and (PAS). X 2000

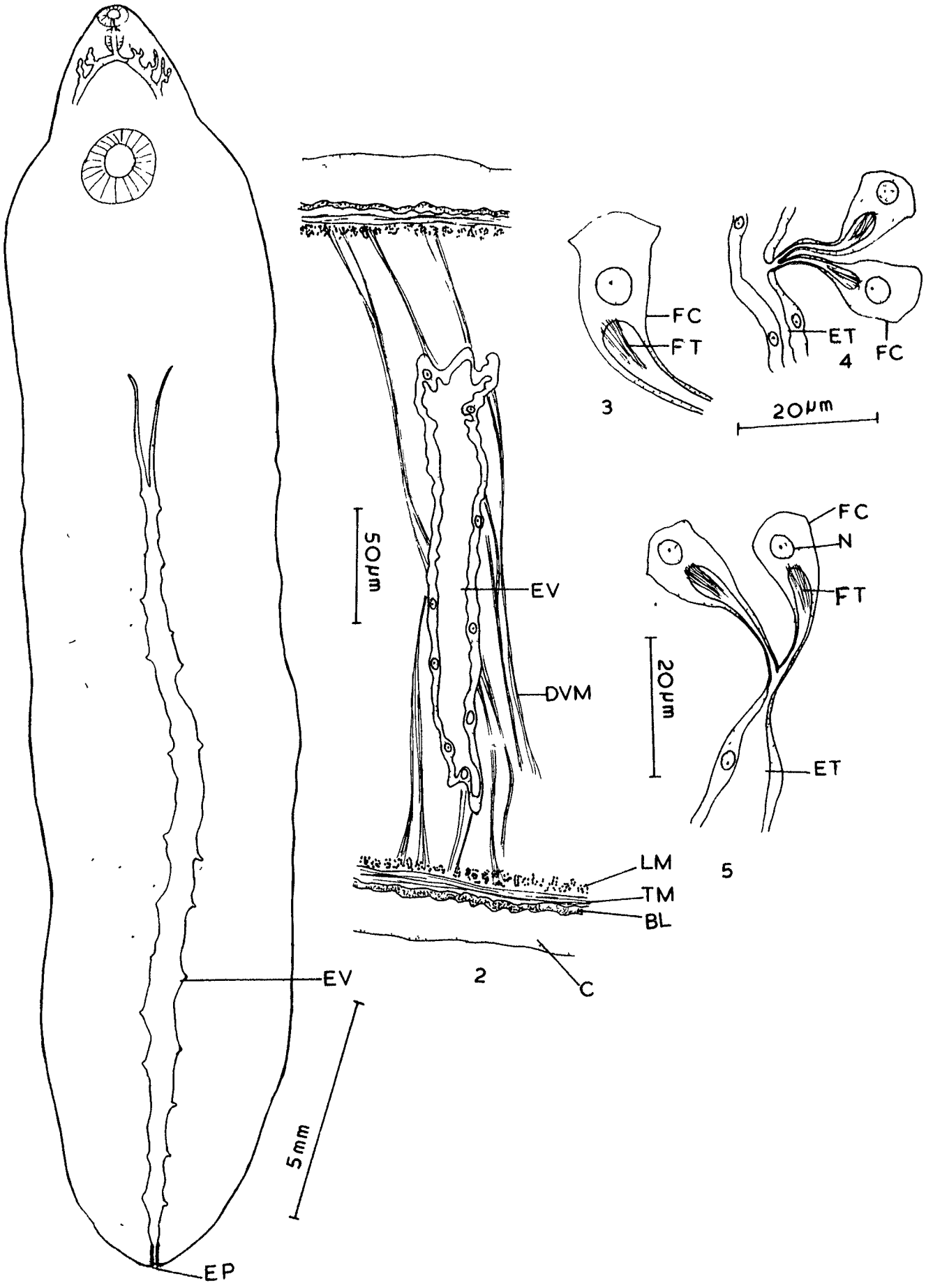

EV
EP
5mm
50µm
EV
DVM
LM
TM
BL
C
2
FC
FT
3
ET
FC
4
20µm
FC
N
FT
20µm
ET
5

PLATE XII

PLATE XIII

Fig. 1. F. gigantica : Cirrus & Cirrus sac through reconstruction, frontal sections stained with Acid Solochrome Cyanine. X 600

Fig. 2. F. gigantica : Prostate gland cells, frontal section stained with (H & E). X 2000

Fig. 3. F. gigantica : Mehlis' gland cells - I, frontal section stained with (H & E). X 2000

Fig. 4. F. gigantica : Mehlis' gland cells - II, frontal section stained with (H & E). X 2000

Fig. 5. F. gigantica : Mehlis' gland complex through reconstruction, frontal sections stained with (H & E). X 600

Fig. 6. F. gigantica : Proximal uterus, frontal section stained with (H & E). X 1000

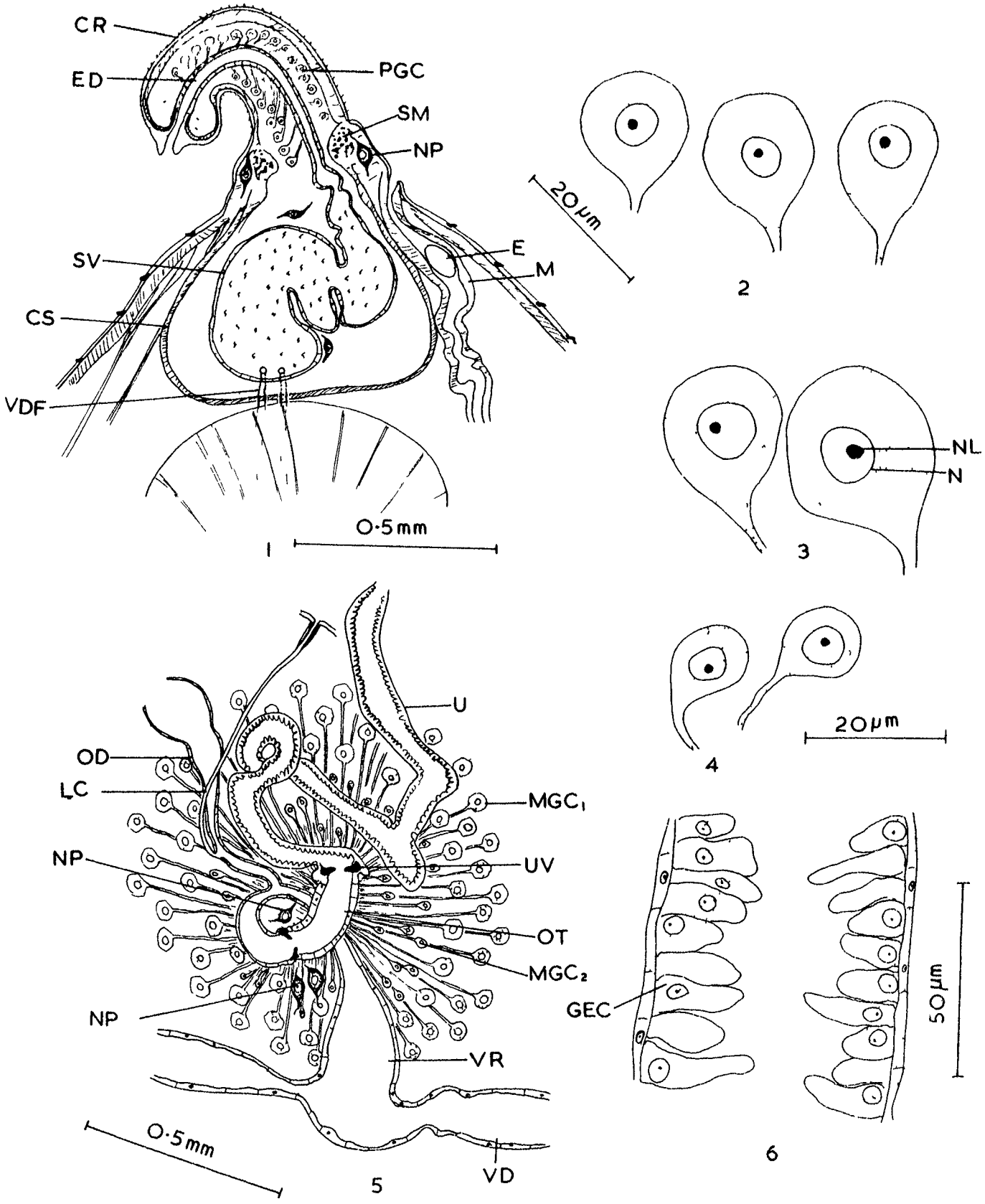

CR
ED
PGC
SM
NP
SV
E
M
CS
VDF
0·5mm
1
20µm
2
NL
N
3
4
U
OD
LC
MGC1
NP
UV
OT
MGC2
NP
VR
0·5mm
VD
5
GEC
50µm
6

PLATE XIII

PLATE XIV

Fig. 1-8. F. gigantica : Stages of spermatogenesis, frontal sections stained with (H & E). X 2000
1, lining of tubule; 2, single germinal cell;
3, tetrad of tertiary spermatogonia;
4, 8-cell morula of primary spermatocytes;
5, 8-cell morula of primary spermatocytes after growth; 6, reduction division (meta phase);
7, 16-cell morula of secondary spermatocytes;
8, 32-cell morula of early spermatids.

Fig. 9-10. F. gigantica : Stages of spermateleosis, frontal section stained with (H & E). X 2000
9, young spermatids; 10, late spermatids

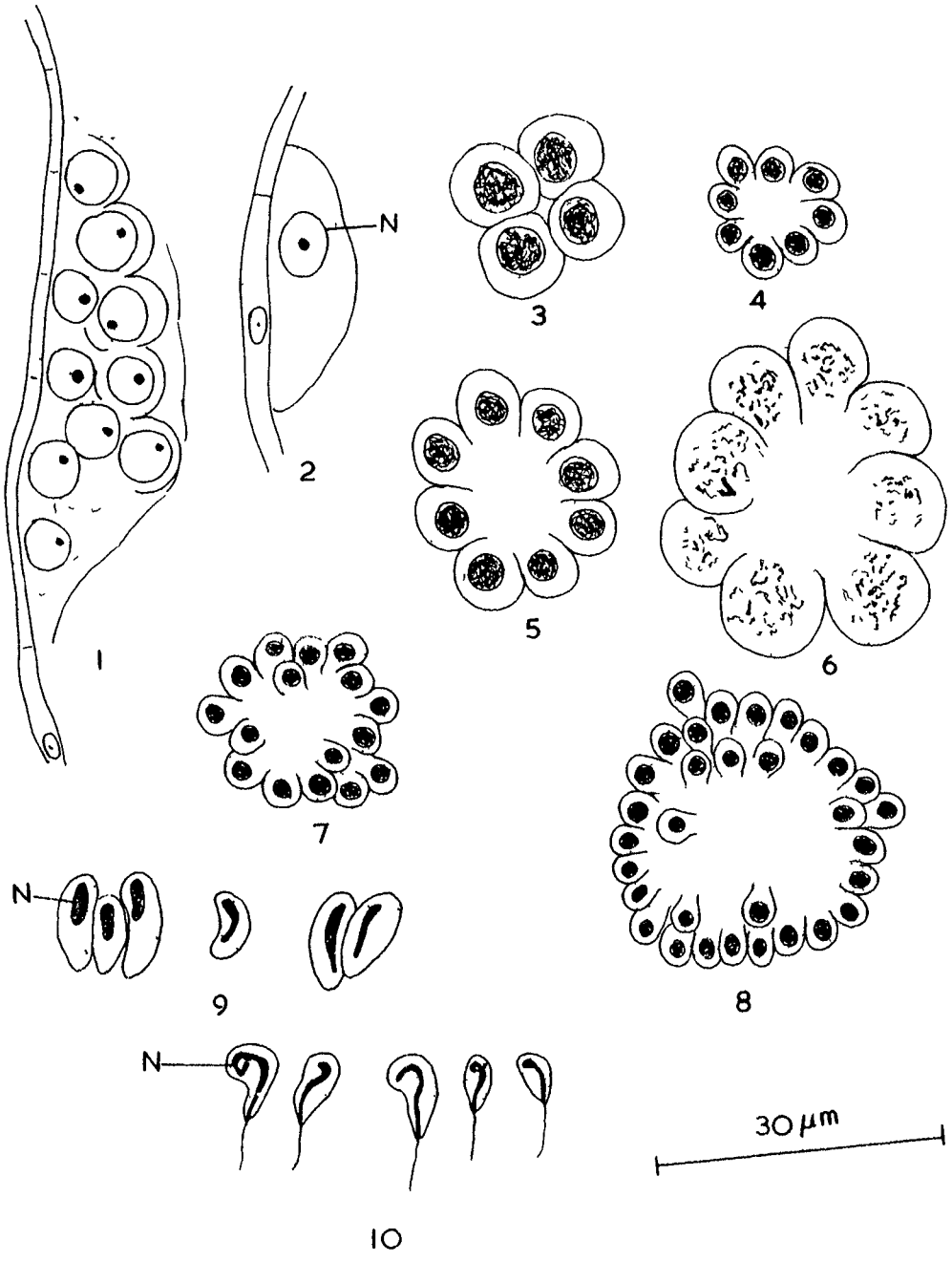


PLATE XIV

PLATE XV

Fig. 1. F. gigantica : Primary oogonia, frontal section stained with Heidenhain's Azan. X 2000

Fig. 2. F. gigantica : Mature oocytes, frontal section stained with Heidenhain's Azan. X 2000

Fig. 3. F. gigantica : Nurse cells in vitelline follicles, frontal section stained with (H & E). X 2000

Fig. 4. F. gigantica : Immature vitelline cell, frontal section stained with Heidenhain's Azan. X 2000

Fig. 5-7. F. gigantica : Mature vitelline cells, frontal sections stained with(H & E). X 2000

Fig. 8. F. gigantica : Vitelline cell from proximal uterus, frontal section stained with (PAS). X 2000

Fig. 9-10. F. gigantica : Eggs from proximal uterus, frontal section stained with (PAS). X 600

Fig. 11. F. gigantica : Fully formed egg from distal uterus, frontal section stained with (PAS). X 600

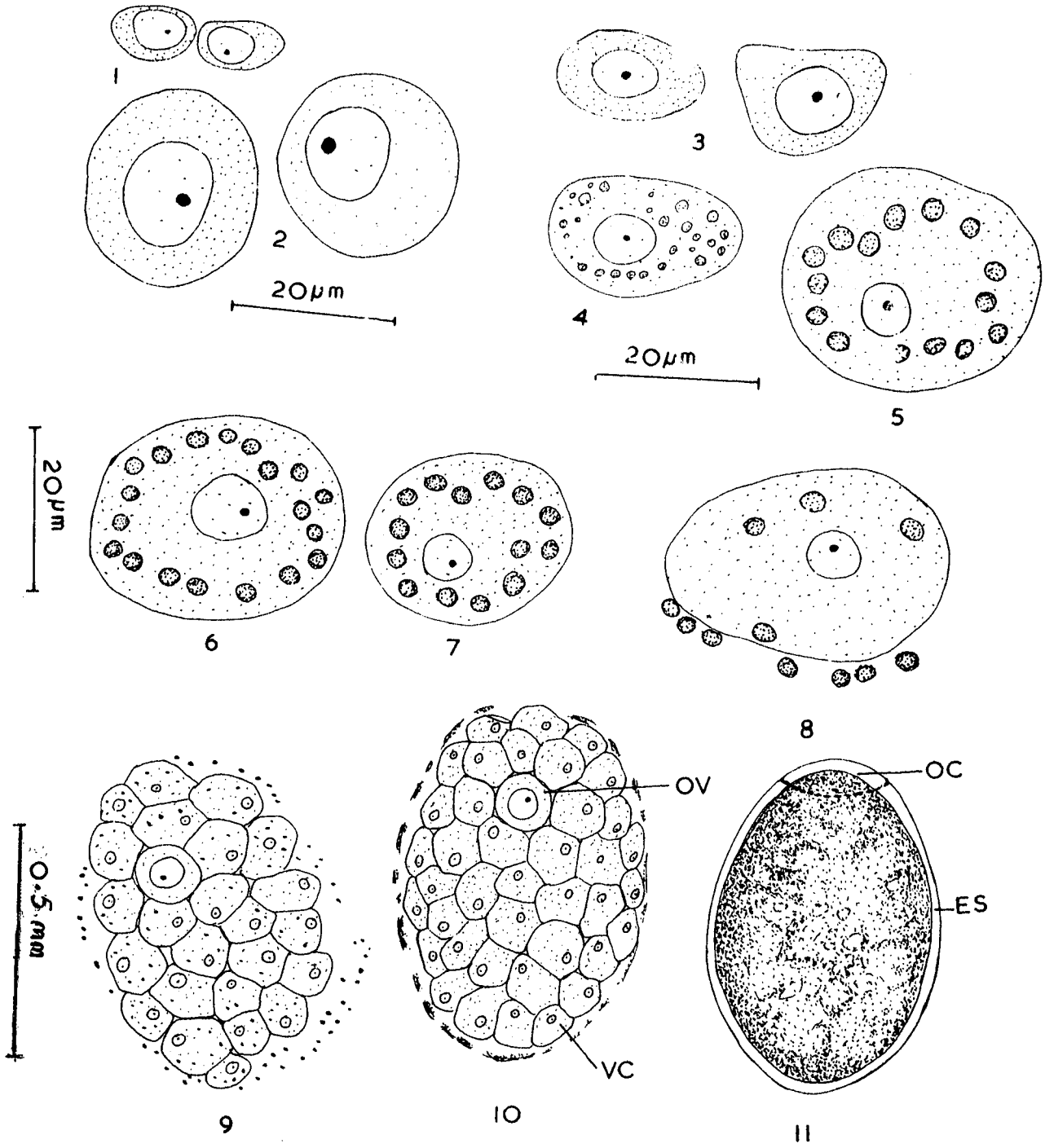


PLATE XV

PLATE XVI

Fig. 1. F. gigantica : Frontal section through tegument and parenchyma (anterior) stained with (PAS) X 100

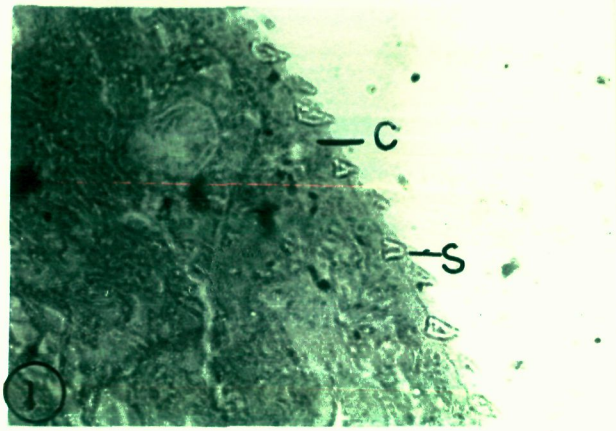
Fig. 2. F. gigantica : Frontal section through peripheral musculature stained with Best's Carmine. X 400

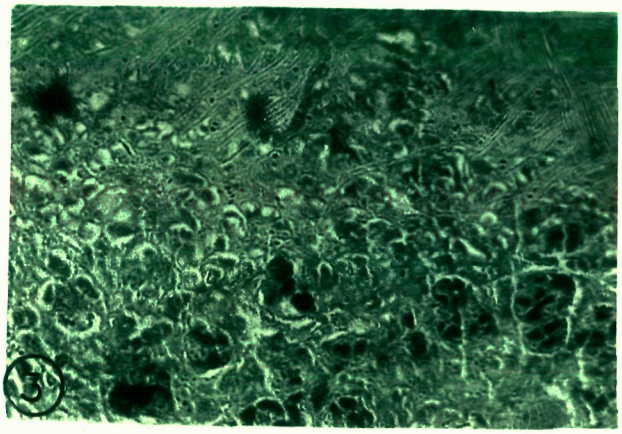
Fig. 3. F. gigantica : Frontal section through hypodermal cells stained with Best's Carmine. X 400

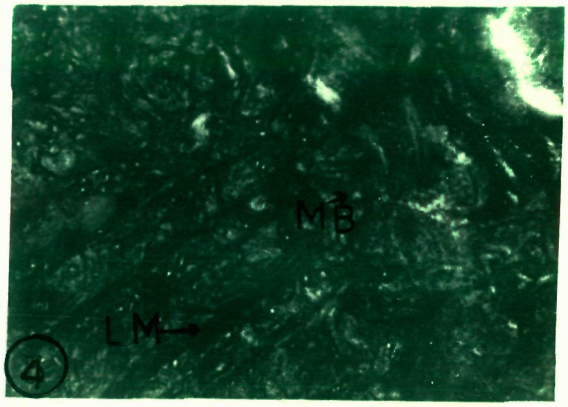
Fig. 4. F. gigantica : Frontal section through dorsoventral muscles stained with Best's Carmine. X 400

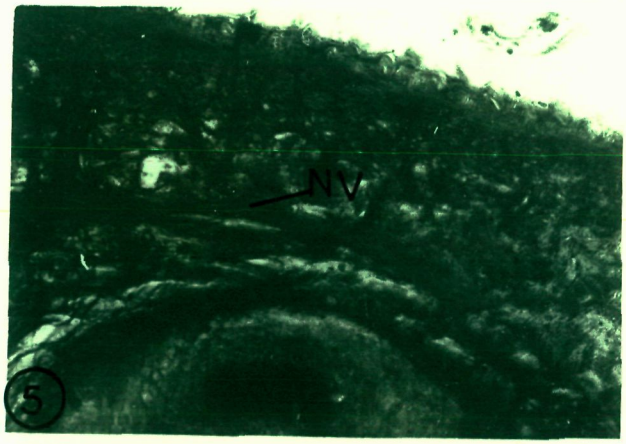
Fig. 5. F. gigantica : Frontal section through antero-lateral region, stained with Sudan black B. X 400

Fig. 6. F. gigantica : Frontal section through superficial cuticle, stained with Acetone Sudan black B. X 400









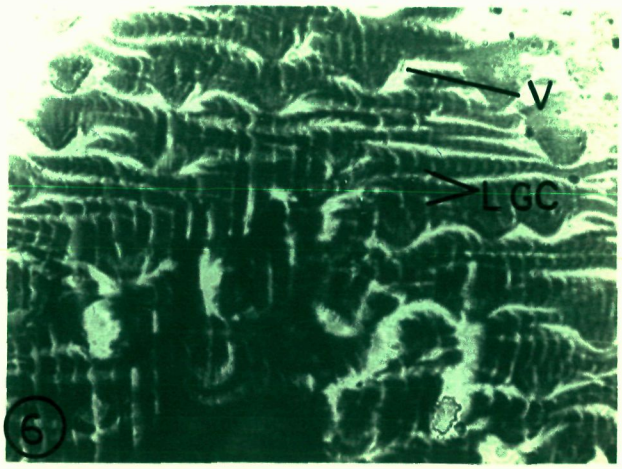


PLATE XVI

PLATE XVII

Fig. 1. F. gigantica : Frontal section through tegument (anterior), stained with Mercury Bromophenol blue. X 100 X 100.

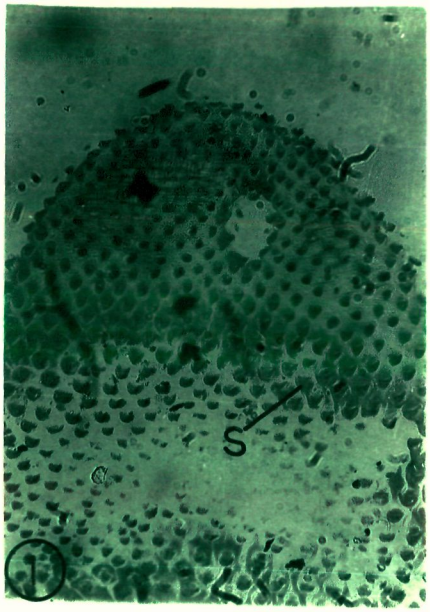
Fig. 2. F. gigantica : Frontal section tegument and intestinal caeca, stained with Alcian blue. X 400

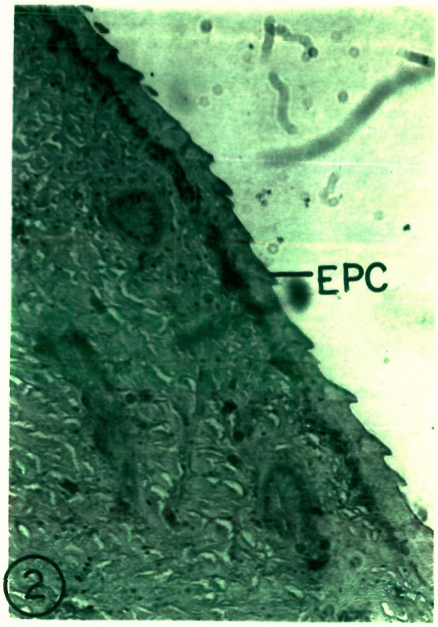
Fig. 3. F. gigantica : Frontal section through peripheral muscles, stained with (H & E). X 100

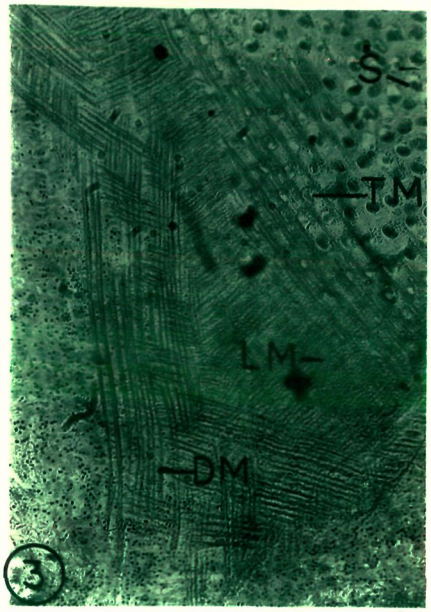
Fig. 4. F. gigantica : Frontal section through tegument, stained with Pyronin Y & Methyl green. X 400

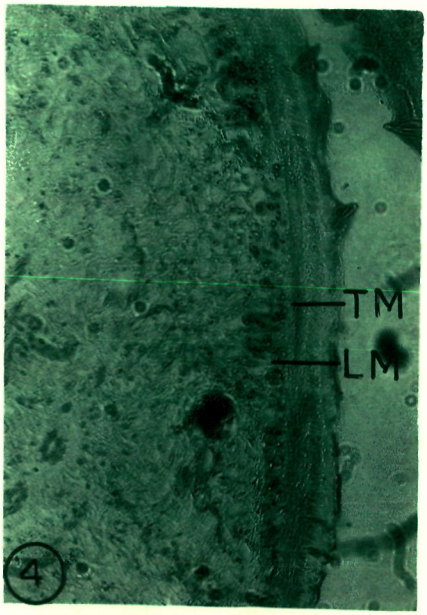
Fig. 5. F. gigantica : Transverse section through tegument, stained with Heidenhain's Azan. X 400

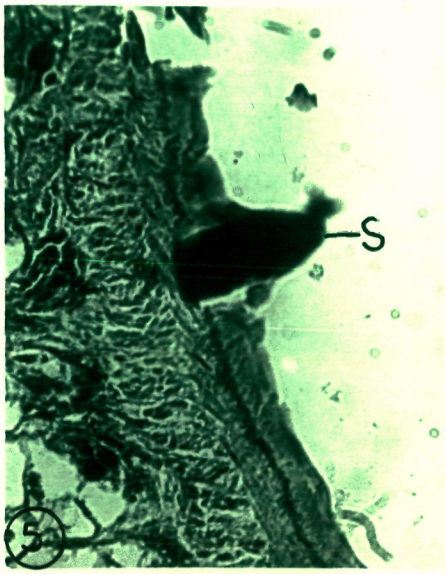
Fig. 6. F. gigantica : Frontal section through tegument, stained with Sudan Black B. X 400











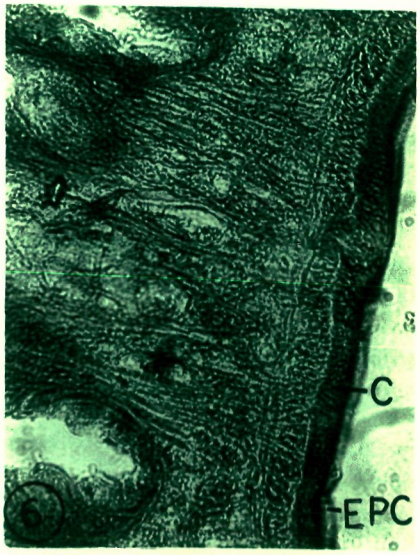


PLATE XVII

PLATE XVIII

Fig. 1. F. gigantica : Frontal section through tegument, stained with (PAS). X 400

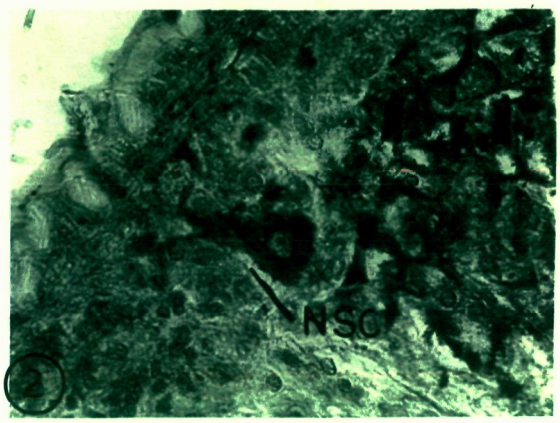
Fig. 2. F. gigantica : Frontal section through tegument, stained with (PAS). X 400

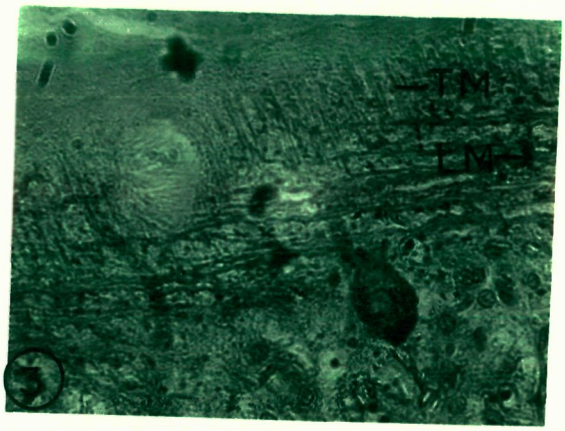
Fig. 3. F. gigantica : Frontal section through tegument (hypodermal region), stained with (PAS). X 400

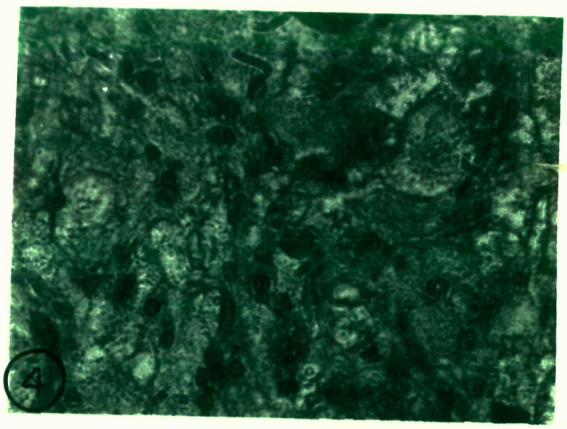
Fig. 4. F. gigantica : Frontal section through hypodermal region, stained with (PAS). X 400

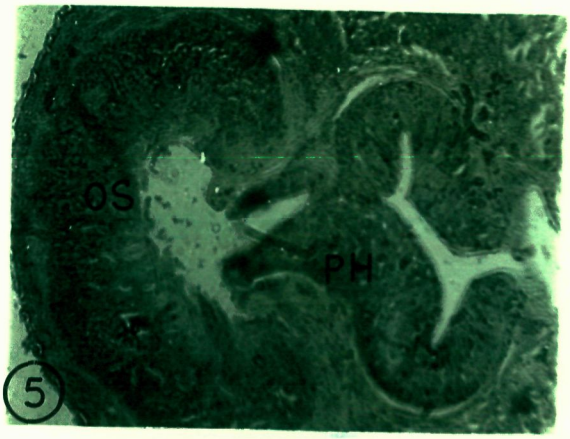
Fig. 5. F. gigantica : Frontal section through oesophagus and pharynx, stained with Best's Carmine. X 100

Fig. 6. F. gigantica : Frontal section through oesophagus, stained with Acetone Sudan black B. X 100









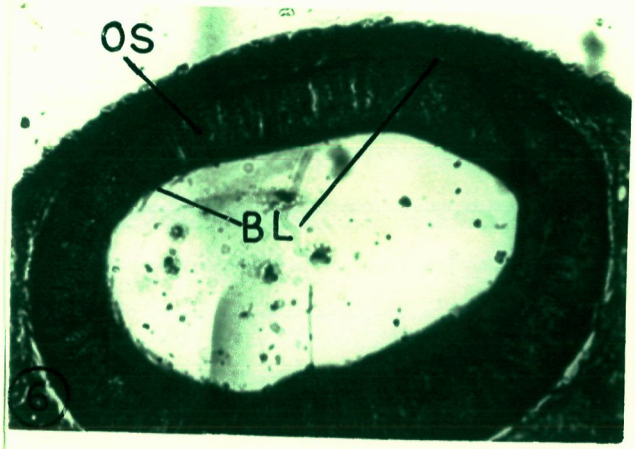


PLATE XVIII

PLATE XIX

Fig. 1. F. gigantica : Frontal section through oesophagus and pharynx, stained with Acid Solochrome Cyanin. X 100

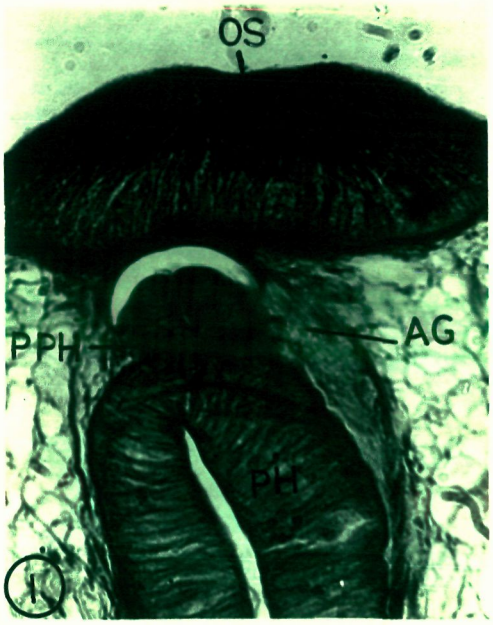
Fig. 2. F. gigantica : Frontal section through excretory pore, stained with Acetone Sudan black B. X 100

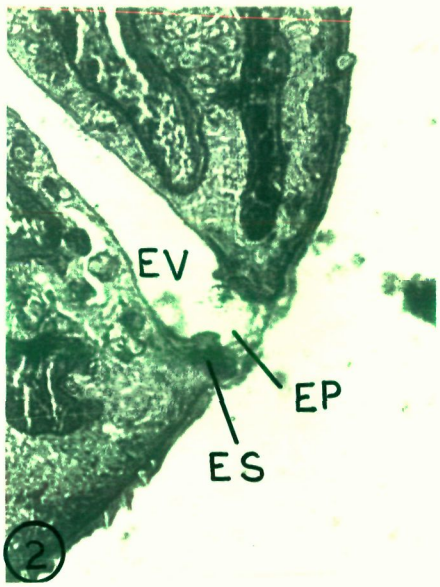
Fig. 3. F. gigantica : Frontal section through oesophagus and pharynx, stained with (H & E). X 100

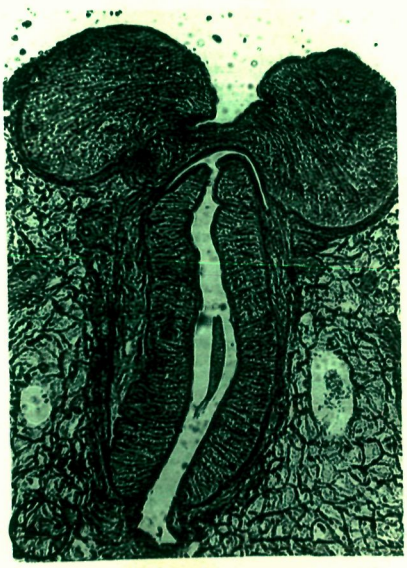
Fig. 4. F. gigantica : Frontal section through anterior region, stained with Sudan black B. X 100

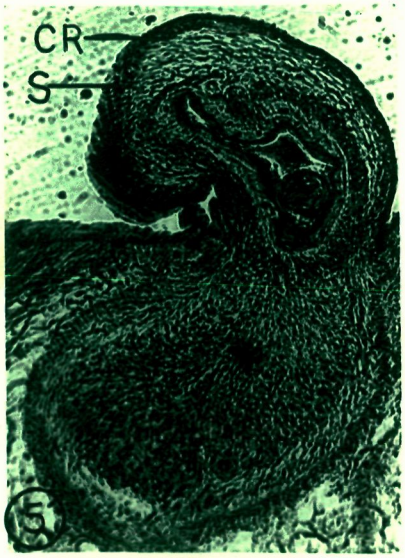
Fig. 5. F. gigantica : Frontal section through cirrus, stained with Sudan black B. X 100

Fig. 6. F. gigantica : Frontal section through excretory vesicle, stained with Sudan black B. X 400









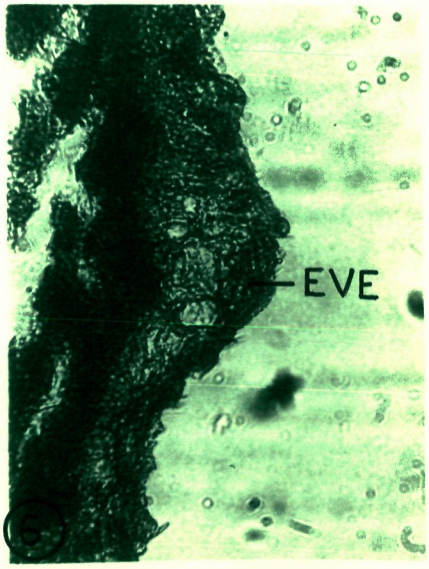


PLATE XIX

PLATE XX

Fig. 1. F. gigantica : Frontal section through ventral sucker and cirrus sac, stained with Acetone Sudan black B. X 100

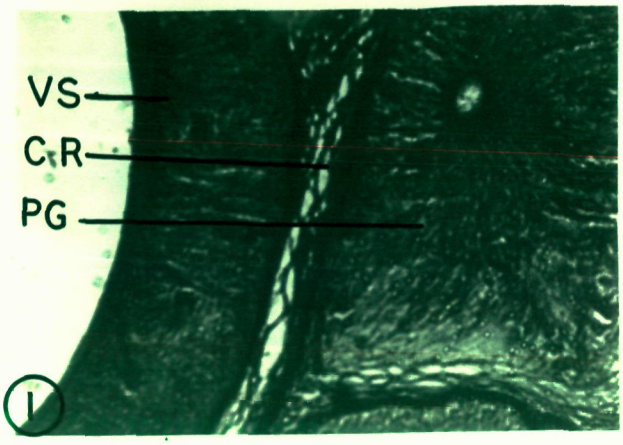
Fig. 2. F. gigantica : Frontal section through ventral sucker, stained with Pyronin Y & Methyl green. X 100

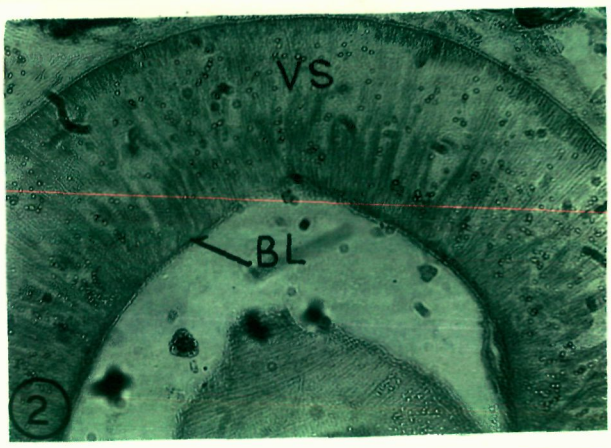
Fig. 3. F. gigantica : Frontal section through adpharyngeal region, stained with Acetone Sudan black B. X 100

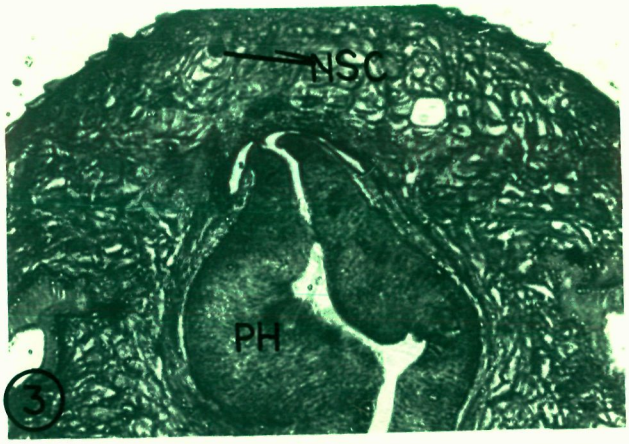
Fig. 4. F. gigantica : Frontal section through oesophagus and pharynx, stained with (PAS). X 100

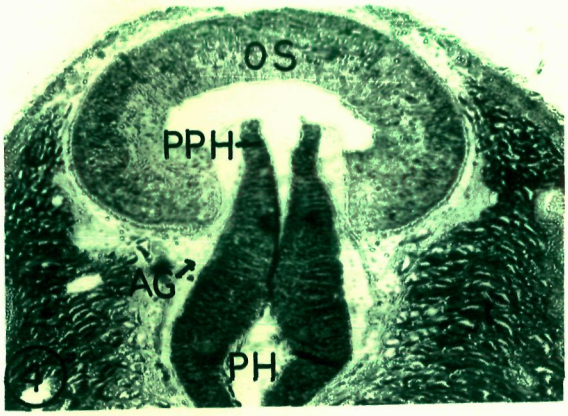
Fig. 5. F. gigantica : Frontal section through ventral sucker, stained with Acid Solochrome Cyanine. X 400

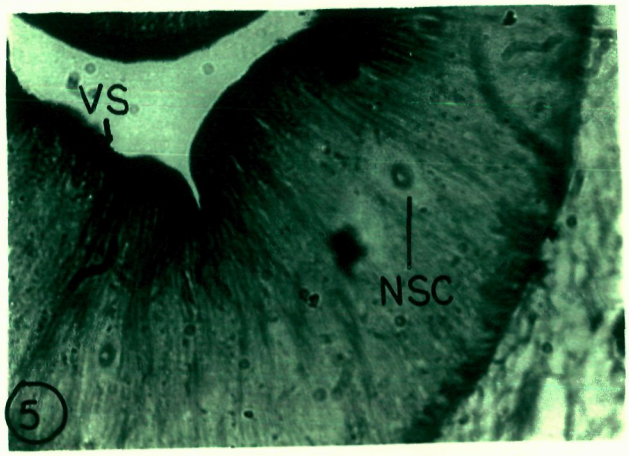
Fig. 6. F. gigantica : Frontal section through cirrus sac and Metraterm, stained with Acid Solochrome Cyanine. X 100











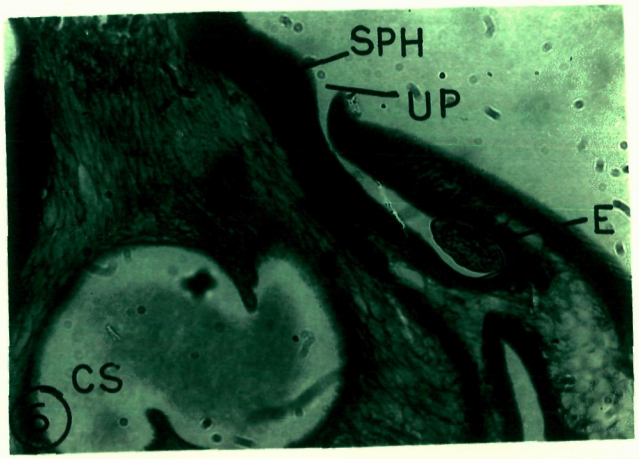


PLATE XX

PLATE XXI

Fig. 1. F. gigantica : Frontal section through ventral sucker and uterus, stained with Acetone Sudan black B. X 100

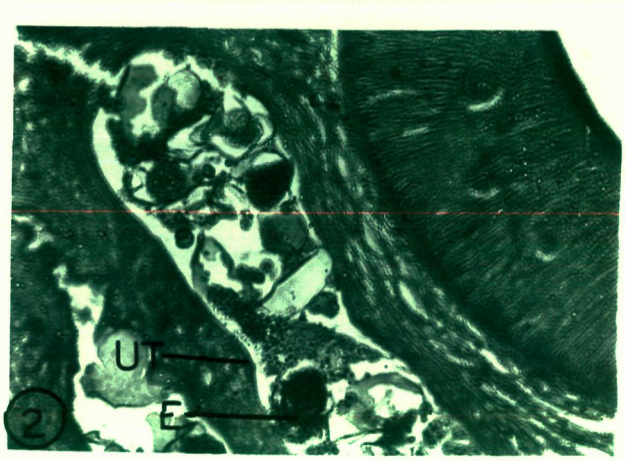
Fig. 2. F. gigantica : Frontal section through ventral sucker and uterus, stained with Mercury Bromophenol blue. X 100

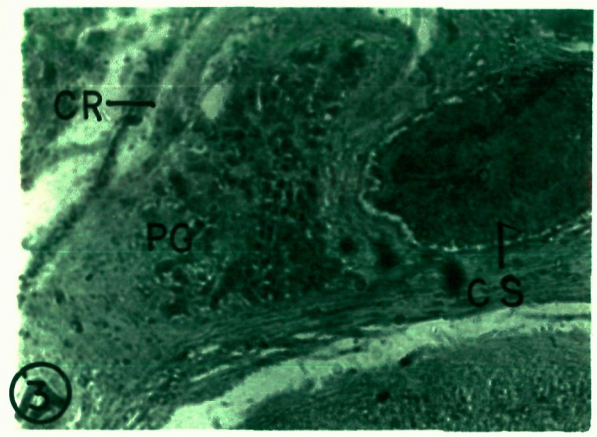
Fig. 3. F. gigantica : Frontal section through cirrus sac, stained with (PAS). X 100

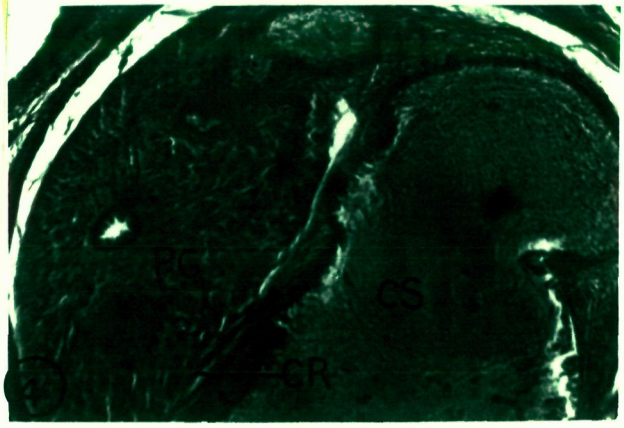
Fig. 4. F. gigantica : Frontal section through cirrus sac, stained with Acetone Sudan black B. X 100

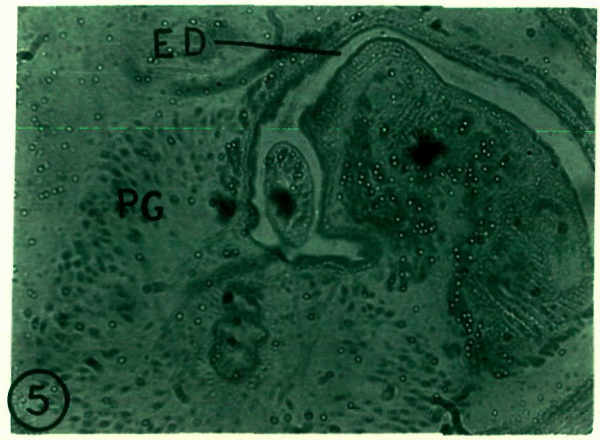
Fig. 5. F. gigantica : Frontal section through cirrus, stained with Pyronin Y & Methyl green. X 100

Fig. 6. F. gigantica : Frontal section through cirrus, stained with (PAS). X 100









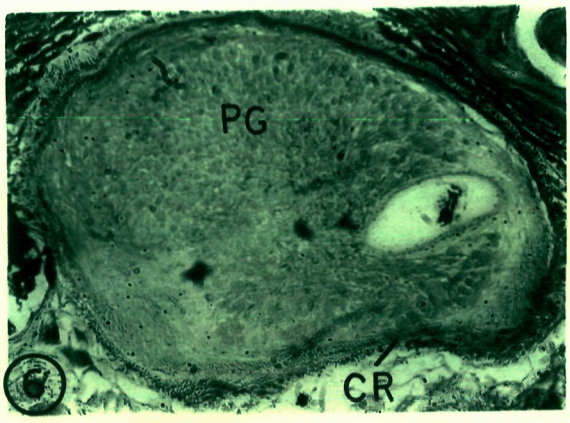


PLATE XXI

PLATE XXII

Fig. 1. F. gigantica : Frontal section through uterus, stained with Best's Carmine. X 400

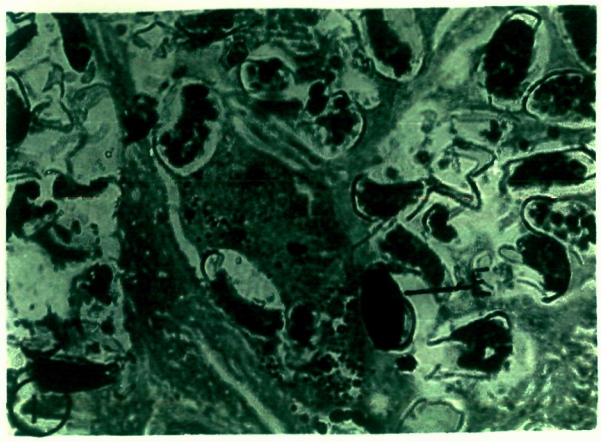
Fig. 2. F. gigantica : Frontal section through uterus, stained with Best's Carmine. X 400

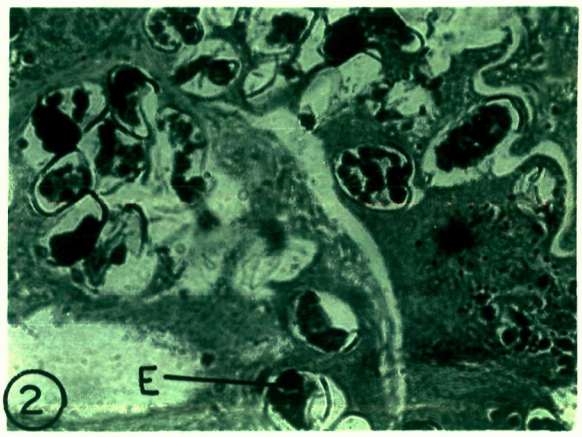
Fig. 3. F. gigantica : Frontal section through cirrus sac, stained with Pyronin Y & Methyl green. X 100

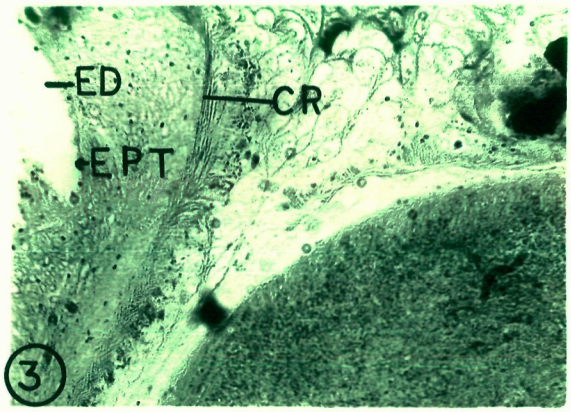
Fig. 4. F. gigantica : Frontal section through cirrus sac, stained with Pyronin Y & Methyl green. X 100

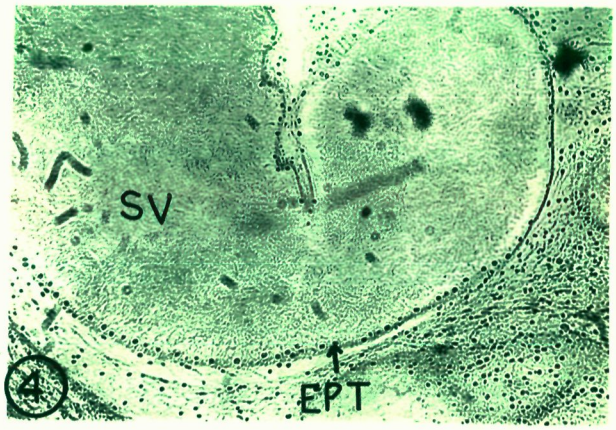
Fig. 5. F. gigantica : Frontal section through Mehlis' gland complex, stained with (PAS). X 100

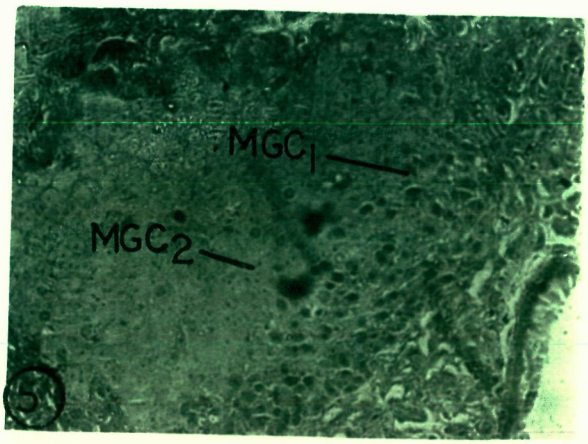
Fig. 6. F. gigantica : Frontal section through Mehlis' gland complex, stained with Acetone Sudan black B. X 100











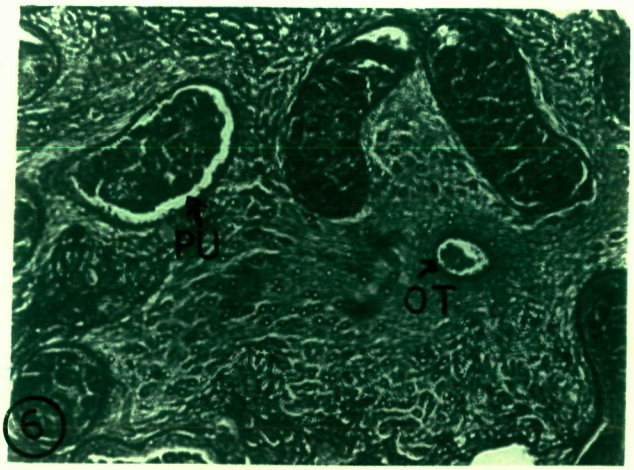


PLATE XXII

PLATE XXIII

Fig. 1. F. gigantica : Frontal section through Mehlis' gland complex, stained with Mercury Bromophenol blue. X 100

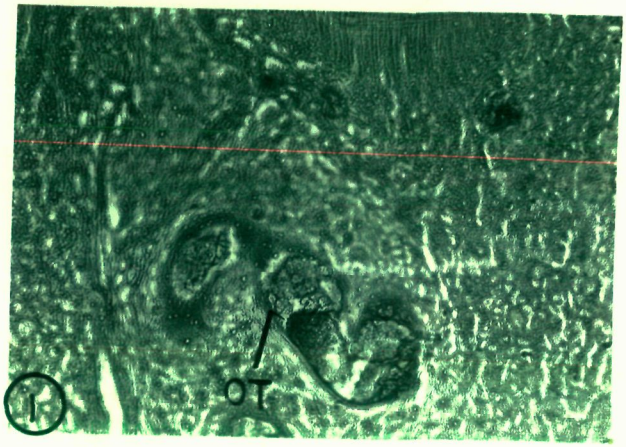
Fig. 2. F. gigantica : Frontal section through vitellaria, stained with Pyronin Y & Methyl green. X 100

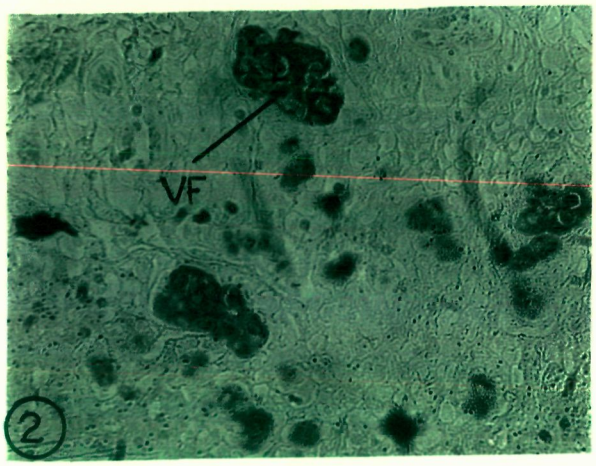
Fig. 3. F. gigantica : Frontal section through Mehlis' gland complex, stained with Best's Carmine. X 100

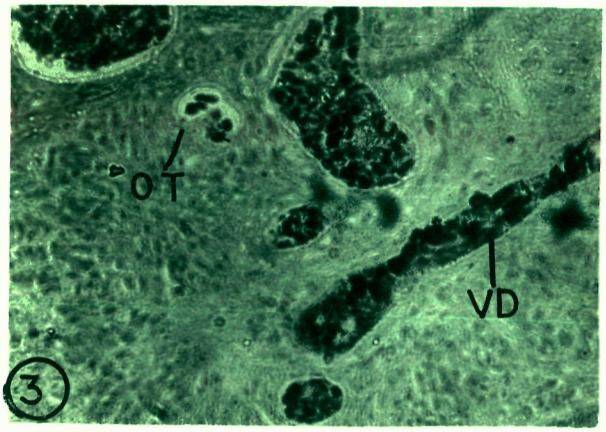
Fig. 4. F. gigantica : Frontal section through Mehlis' gland complex, stained with Pyronin Y & Methyl green. X 100

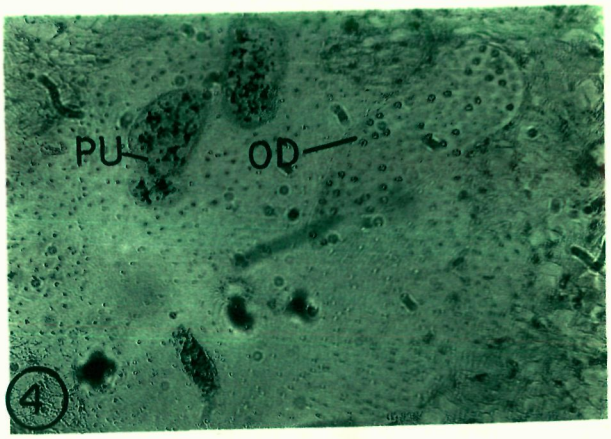
Fig. 5. F. gigantica : Frontal section through Mehlis' gland complex, stained with Acid Solochrome Cyanine. X 100

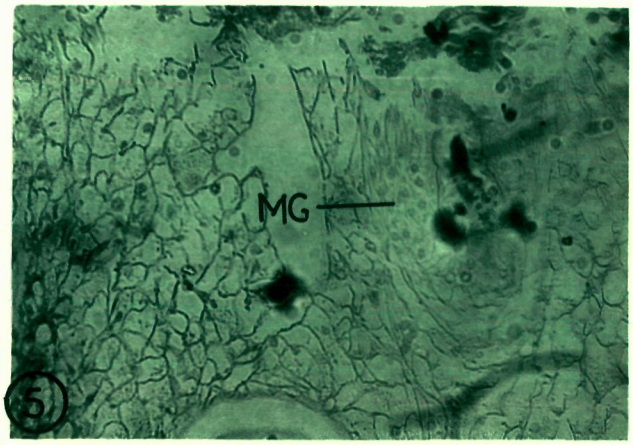
Fig. 6. F. gigantica : Frontal section through Mehlis' gland complex, stained with Acetone Sudan black B. X 100











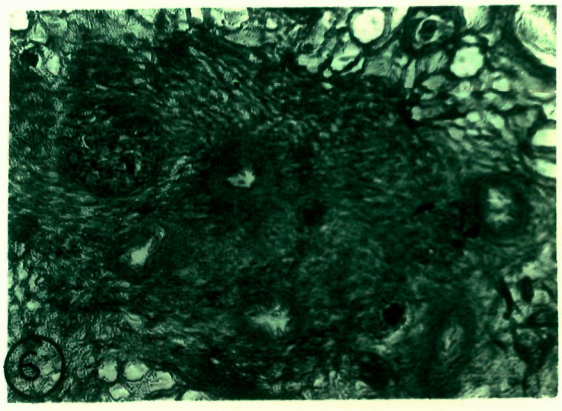


PLATE XXIII

PLATE XXIV

Fig. 1. F. gigantica : Frontal section through ovary, stained with Acetone Sudan black B. X 100

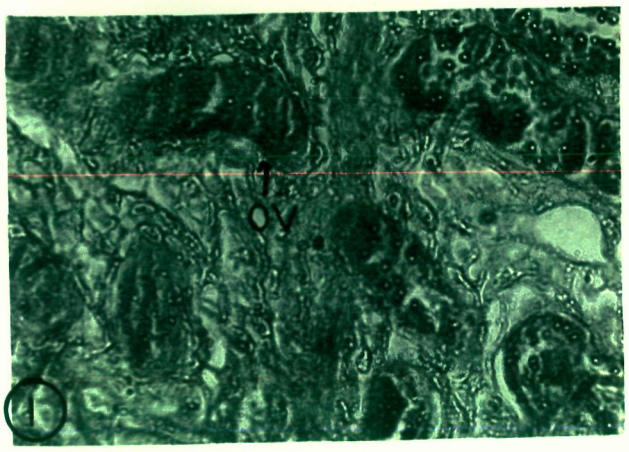
Fig. 2. F. gigantica : Frontal section through ovary stained with Mercury Bromophenol blue. X 100

Fig. 3. F. gigantica : Frontal section through intestinal caeca, stained with Acetone Sudan black B. X 100

Fig. 4. F. gigantica : Frontal section through proximal uterus, stained with (PAS). X 100

Fig. 5. F. gigantica : Frontal section through ovary, stained with (PAS). X 100

Fig. 6. F. gigantica : Frontal section through ovary and uterus, stained with Sudan black B. X 100



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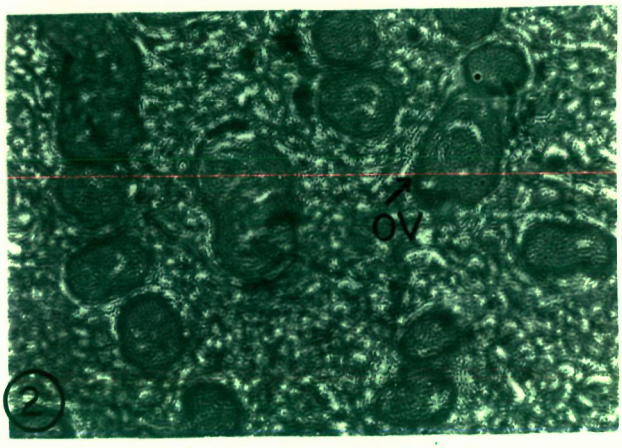

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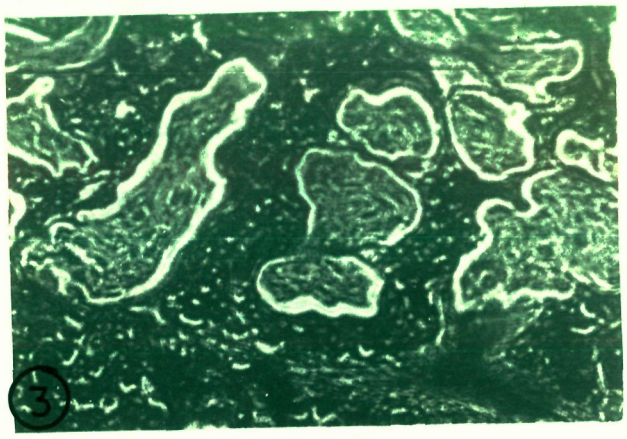

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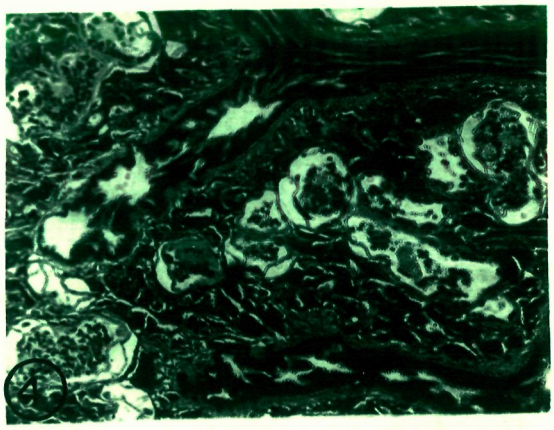

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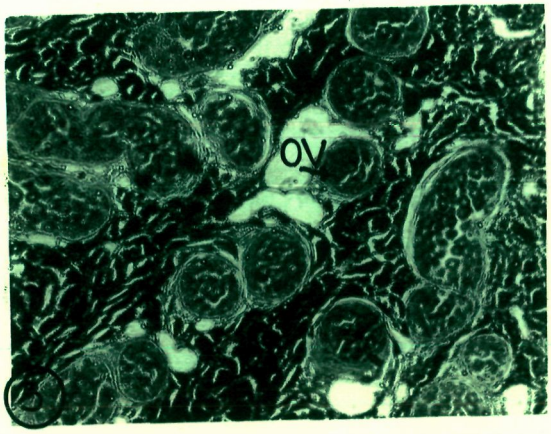

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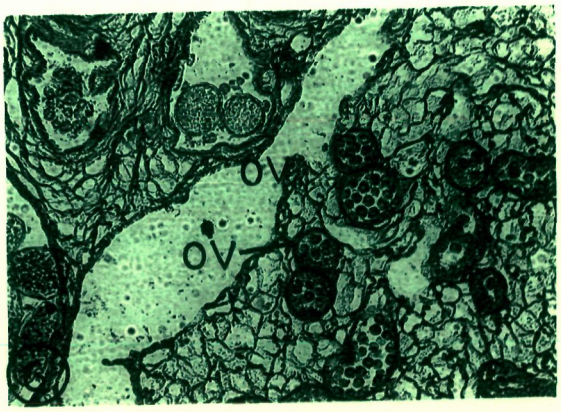


6

PLATE XXIV

PLATE XXV

Fig. 1. F. gigantica : Frontal section through ventral nerve connection, stained with (PAS). X 400

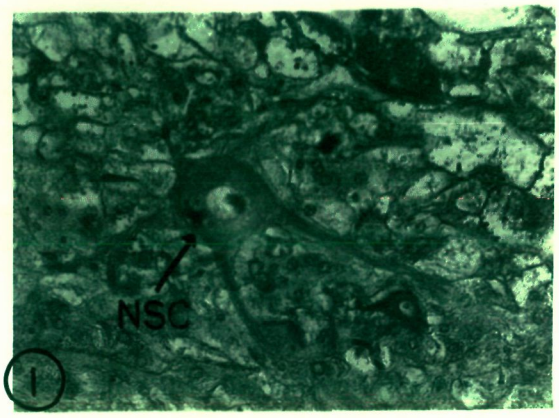
Fig. 2. F. gigantica : Frontal section through adpharyngeal region, stained with Chrome haematoxylin and phloxine. X 400

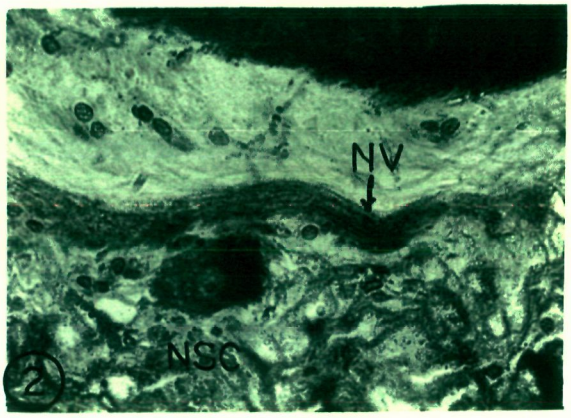
Fig. 3. F. gigantica : Frontal section through ventral nerve, stained with Mercury Bromophenol blue. X 400

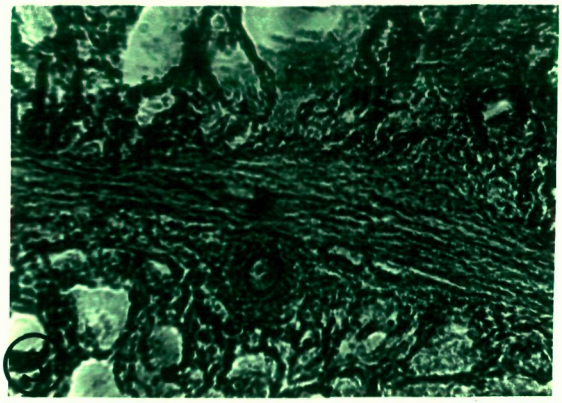
Fig. 4. F. gigantica : Frontal section through ventral nerve, stained with Sudan black B. X 400

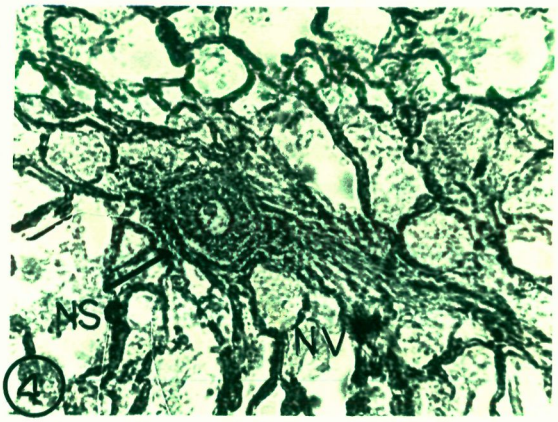
Fig. 5. F. gigantica : Frontal section through anterior ganglia, stained with Chrome haematoxylin and phloxine. X 400

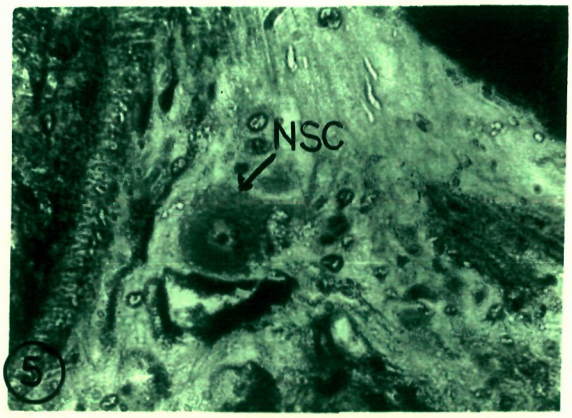
Fig. 6. F. gigantica : Frontal section through ventral sucker, stained with Chrome haematoxylin and phloxine. X 400











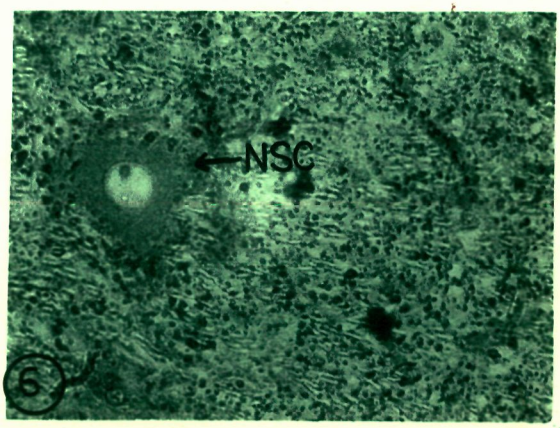


PLATE XXV

PLATE XXVI

Fig. 1. F. gigantica : Transverse section through anterior diagonal muscle, stained with Heidenhain's Azan. X 400

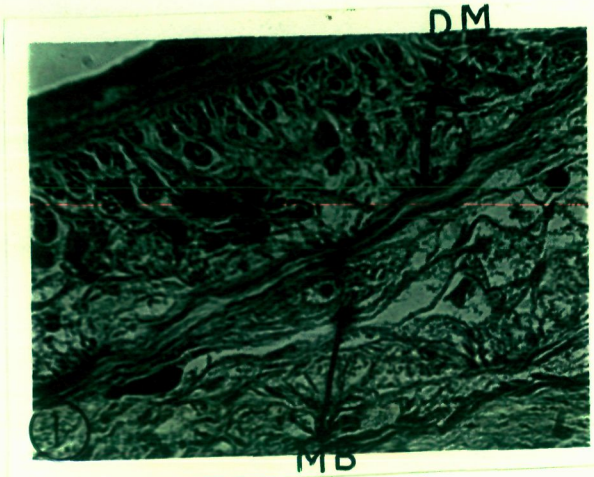
Fig. 2. F. gigantica : Transverse section through ventral nerve, stained with Chrome haematoxylin and Phloxin. X 400

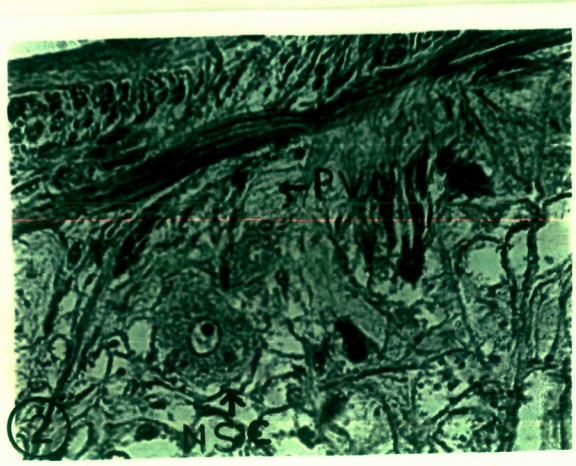
Fig. 3. F. gigantica : Frontal section through adpharyngeal region, stained with Acid Solochrome Cyanine. X 100

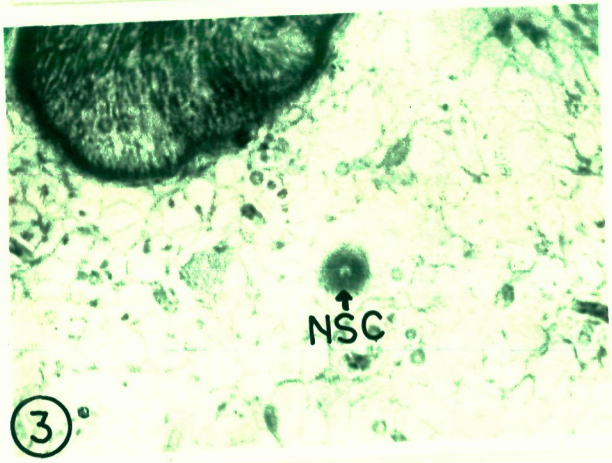
Fig. 4. F. gigantica : Transverse section through ventral nerve, stained with Chrome haematoxylin and Phloxin. X 400

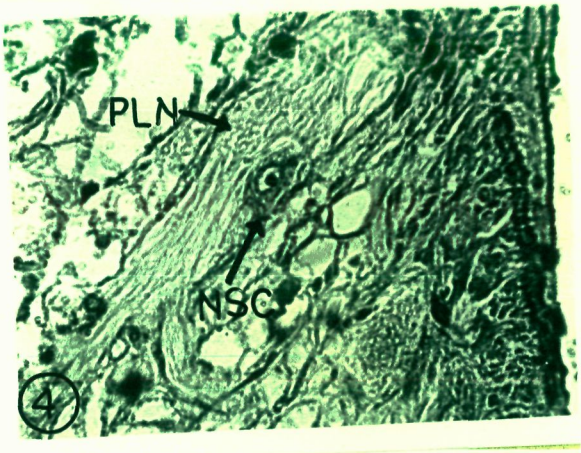
Fig. 5. F. gigantica : Frontal section through ventral nerve, stained with (PAS). X 400

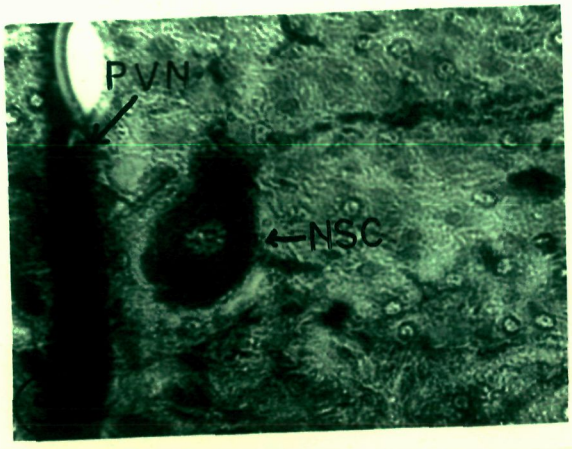
Fig. 6. F. gigantica : Frontal section through posterior (parenchyma) region, stained with Chrome haematoxylin and Phloxin. X 400











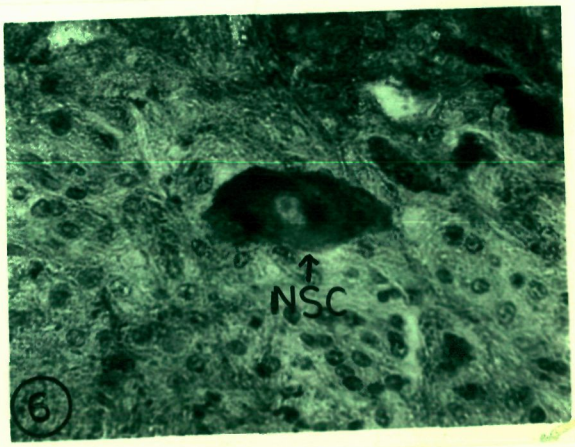


PLATE XXVI

PLATE XXVII

Fig. 1. F. gigantica : Frontal section through posterior (parenchyma) region, stained with Mercury Bromophenol blue. X 400

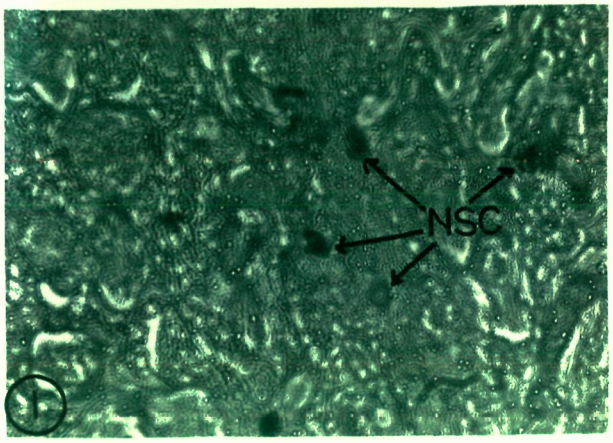
Fig. 2. F. gigantica : Frontal section through intestinal caeca and vitelline follicles, stained with Acid Solochrome Cyanine. X 400

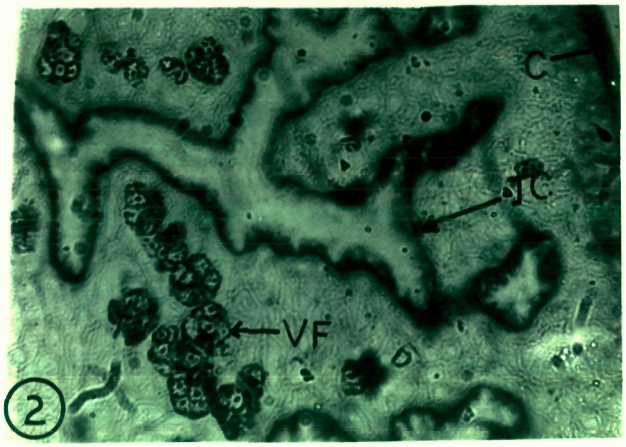
Fig. 3. F. gigantica : Frontal section through Mehlis' gland complex stained with (PAS). X 400

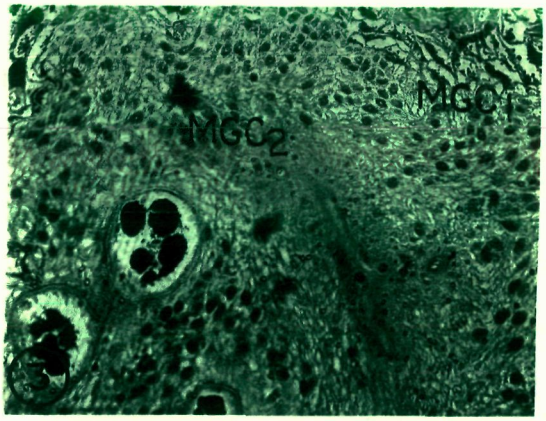
Fig. 4. F. gigantica : Frontal section through ventral sucker, stained with Sudan black B. X 100

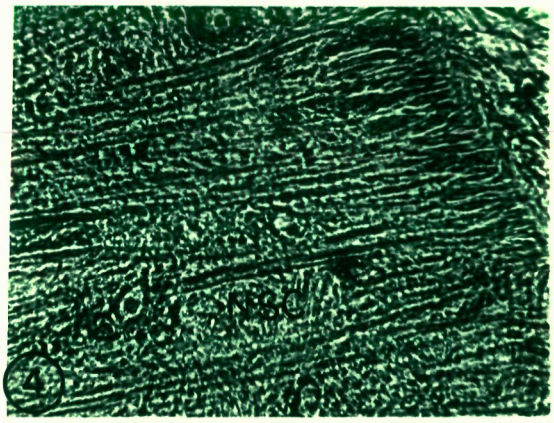
Fig. 5. F. gigantica : Frontal section through intestinal caecum, stained with (PAS). X 400

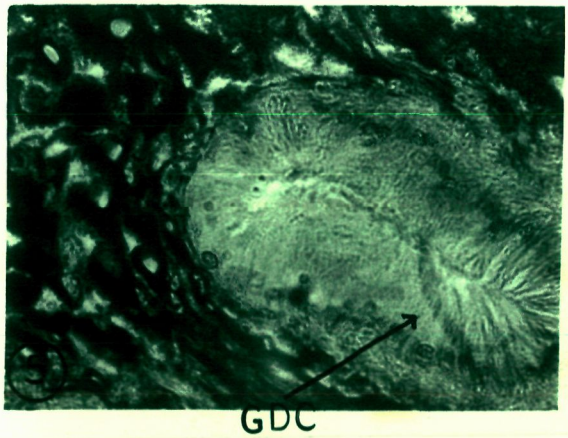
Fig. 6. F. gigantica : Frontal section through intestinal caecum, stained with Sudan black B. X 400











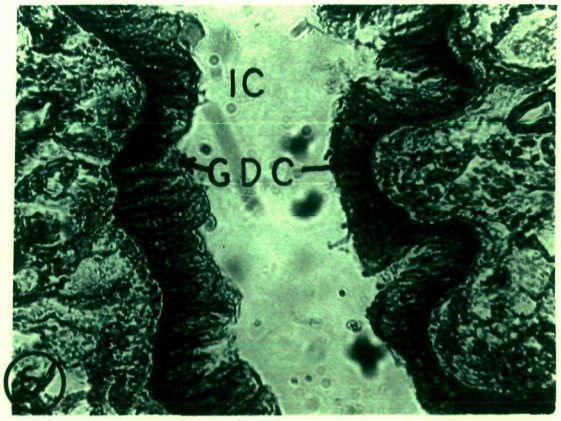


PLATE XXVII

PLATE XXVIII

Fig. 1. F. gigantica : Posterior region, toto mount stained with Bromo Indoxyl acetate. X 100

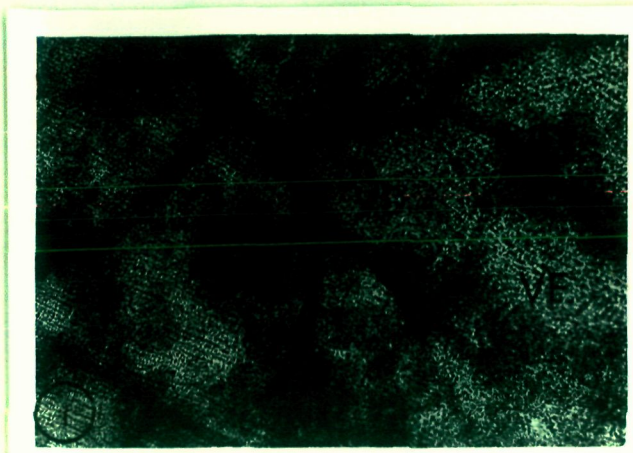
Fig. 2. F. gigantica : Frontal section through lateral vitelline duct, stained with Acid Solochrome Cyanine. X 100.

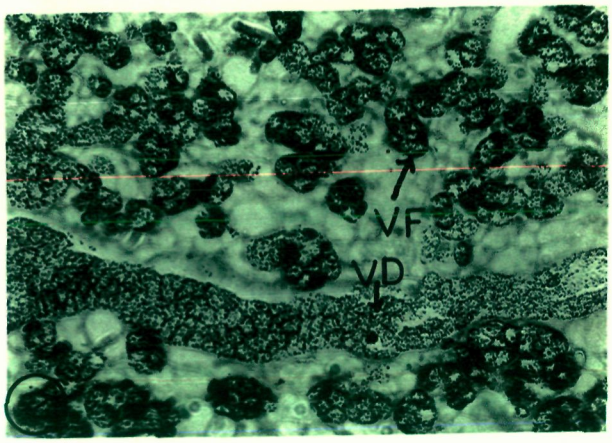
Fig. 3. F. gigantica : Frontal section through vitelline follicles, stained with (PAS). X 400.

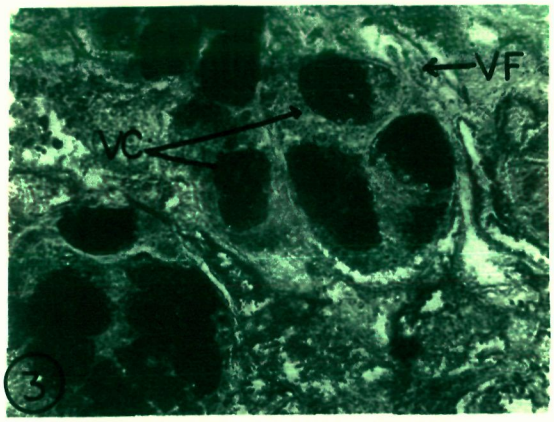
Fig. 4. F. gigantica : Frontal section through proximal uterus, stained with (PAS). X 400.

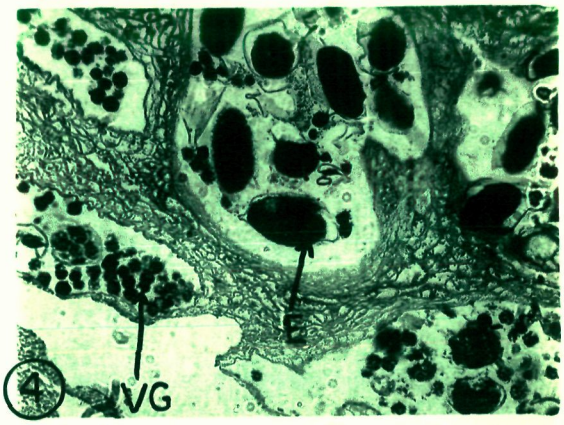
Fig. 5. F. gigantica : Frontal section through vitelline follicles, stained with Mercury Bromophenol blue. X 100.

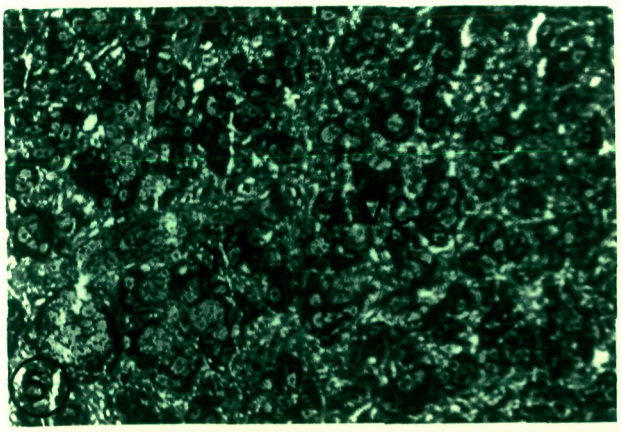
Fig. 6. F. gigantica : Frontal section through vitelline follicles, stained with Sudan black B. X 100.











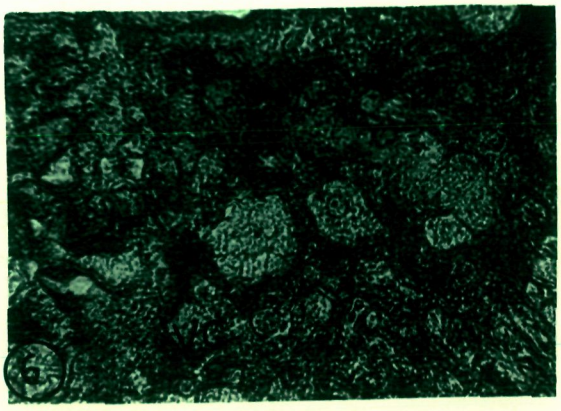


PLATE XXVIII

PLATE XXIX

Fig. 1. *F. gigantica* : Anterior region, *toto* mount stained with Indoxyl acetate. X 100.

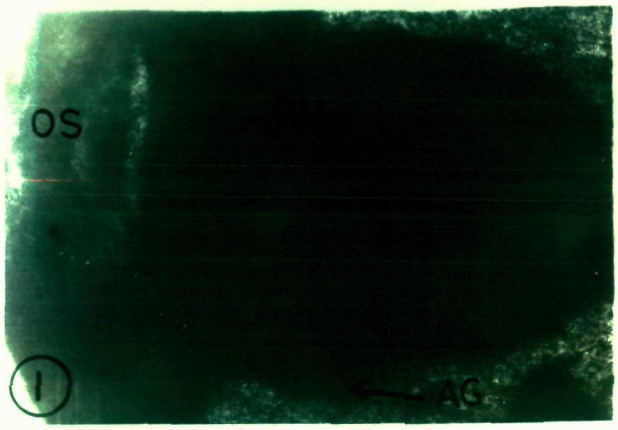
Fig. 2. *F. gigantica* : Anterior region, *toto* mount stained with Acetylthiocholine iodide. X 100.

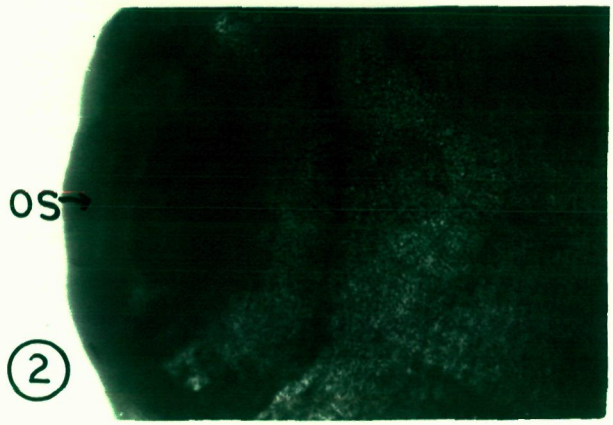
Fig. 3. *F. gigantica* : Posterior region, *toto* mount stained with Indoxyl acetate. X 100

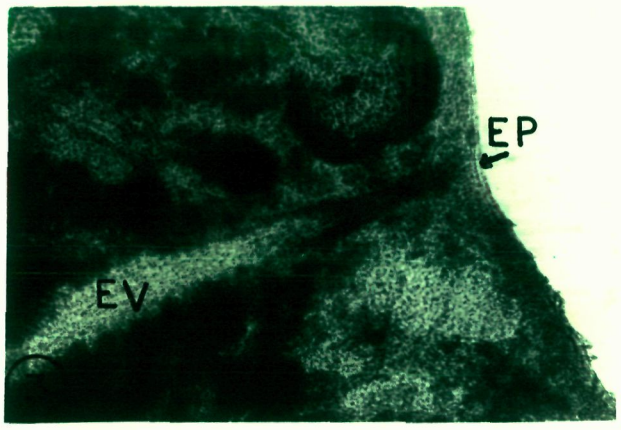
Fig. 4. *F. gigantica* : Posterior region, *toto* mount stained with Acetylthiocholine iodide. X 100.

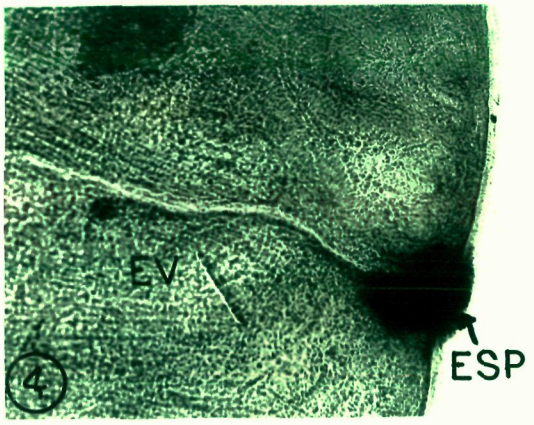
Fig. 5. *F. gigantica* : Acetabular region, *toto* mount stained with Indoxyl acetate. X 25.

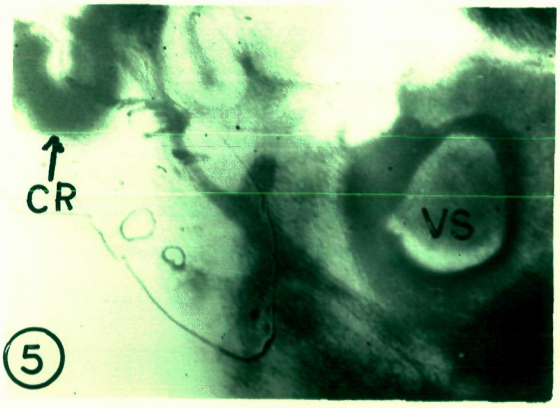
Fig. 6. *F. gigantica* : Anterior region, *toto* mount stained with Indoxyl acetate. X 25.











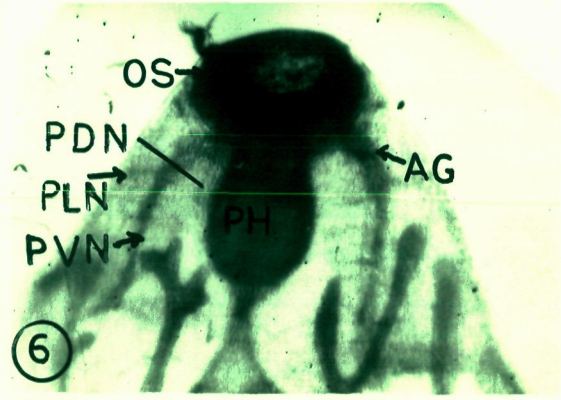


PLATE XXIX

PLATE XXX

Fig. 1. F. gigantica : Lateral vitelline duct region, toto mount stained with Indoxyl acetate. X 100.

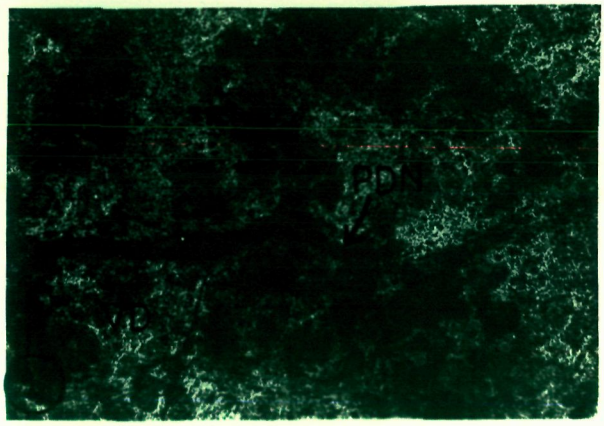
Fig. 2. F. gigantica : Lateral vitelline duct region, toto mount stained with Indoxyl acetate. X 400

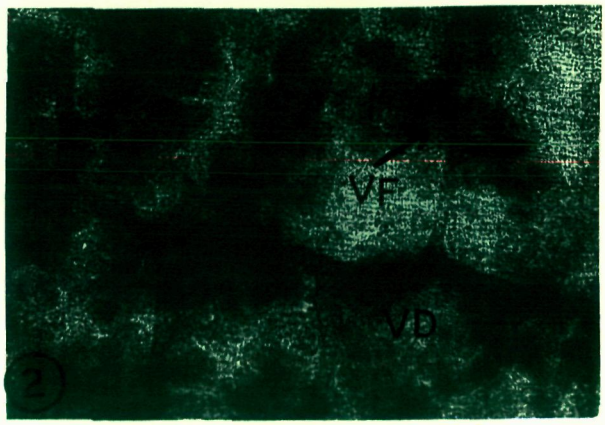
Fig. 3. F. gigantica : Posterior region, toto mount stained with Acetylthiocholine iodide. X 100

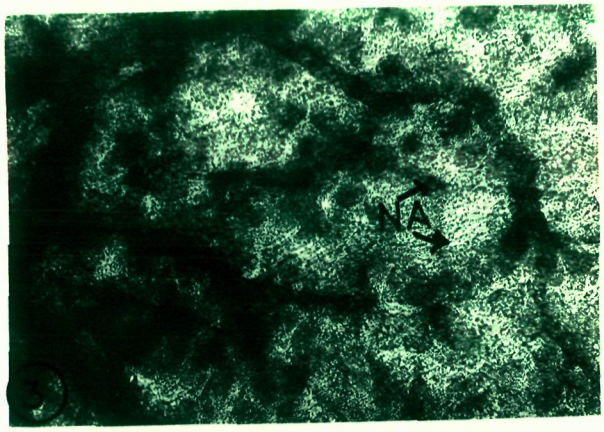
Fig. 4. F. gigantica : Posterior region, toto mount stained with Indoxyl acetate. X 100

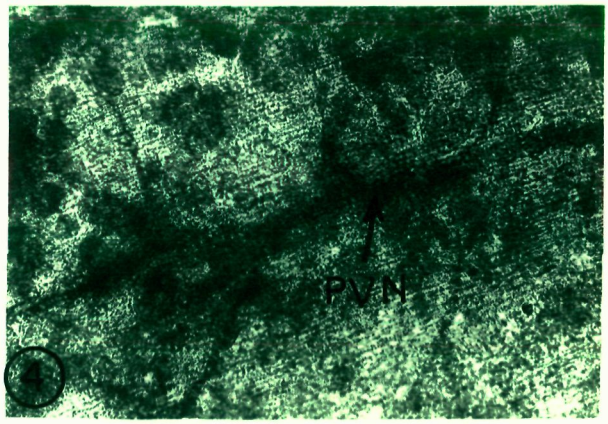
Fig. 5. F. gigantica : Lateral region, toto mount stained with Acetylthiocholine iodide. X 100

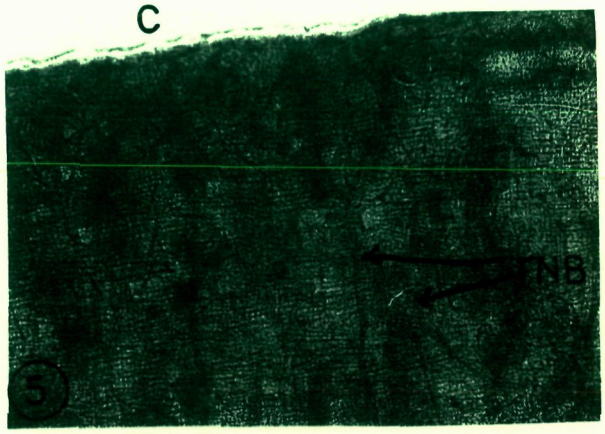
Fig. 6. F. gigantica : Lateral region, toto mount stained with Indoxyl acetate. X 100











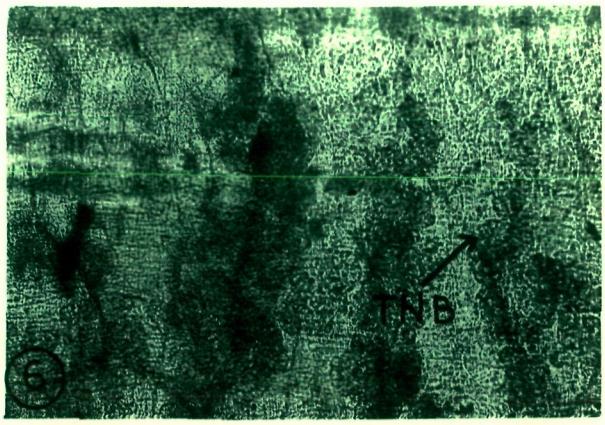


PLATE XXX